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EDITORIAL

From Maiden Blush to Matron's Flush

.apidly changing colour of the skin is always aused by altered flow of blood It has attracted the erest of the layman and the observations are ften evaluated emotionally deadly pale rosy ..lthy cheeks a lovely blushing maiden etc

The clinician has much to learn from such henomena Conditions accompanied by pallor and weating may be very important from therapeutic oints of view When they occur in attacks together ith increase in blood pressure they may indicate e presence of a phaeochromocytoma It has been id that sweating is compulsory but even if it is y common there are examples of this disease ho do not transpire during the attacks

It is well known that the flush area is localized to face and to a V formed part of the skin on the pper frontal part of the thorax in women This is also the area of predilection for the skin lesions in ystemic lupus

Maiden blush is the poetical name for a medical ymptom that is of no diagnostic importance as far as we know but may cause the individual trouble sychologically It deserves its name as it is com on in young females I have seen it last until the ge of 30 years in one woman but when I saw her gain at the age of 45 it had disappeared

The distribution is of course only in the flush area nd it is characteristic that there are a large number f rounded patches sometimes coalescing with a red colour The same type of blush may be een in some diseased conditions in patients who re past the maiden age but as far as my experience oes always in females I have seen it in a woman ged 50 who has suffered from medullary carcinoma r many years I have also seen it in an elderly y who had a very severe hypertension obviously nnected with liberation of some vasoactive subtance It is probable that the enigma of maiden ush will be solved through biochemical studies of ome such case

The blush in young females may be regarded as normal whereas the hot flushes at the menopause really cause their victims very severe discomfort It is not known what percentage of climacteric women suffer from these sensations but it is probable that there are all degrees even if certain persons maintain that they have never noticed any sensations Most disagreeable is the feeling of hotness in the upper part of the body and also that the head feels as if it were going to burst The observer does not notice very remarkable changes even if there is a reddish hue that is spread evenly and does not show any clearcut demarcation line against normal skin Sweat baths are of course also common in the menopause but they seem to be quite independent from the flushes

There is a group of definitely pathological flushes that have great diagnostic importance and therefore should be observed in detail The British cardiologist Sir Maurice Cassidy observed what he called phenomenal flushing in a male patient with pancreatic carcinoma He was so impressed by the symptom that he published a coloured plate of a drawing in the Proc Royal College of Medicine We observed a similar type of flushing in two patients with metastasizing intestinal carcinoid tumours Later a large number of such cases have been published It is surprising that the cutaneous symptoms may vary so much Some patients have rapidly changing patterns of red blue yellow skin all over trunk and extremities They may also show flushing at the same time on the peritoneum and the extremely marked changes in the ballistocardiogram definitely show that they must be flushing all over Together with the flush there is often borborygmi and diarrhoea but no itching

The flush lasts only for some minutes Psychological factors as well as a meal normally the breakfast may induce a flush As the tumour has no innervation it is clearly mediated by humoral factors catecholamines i v are potent flush factors with a delay of some seconds before the acti

principle is liberated Many facts seem to indicate that some kinin perhaps bradykinin is liberated and accounts for the vasomotor symptoms whereas the diarrhoea that is such a common companion is probably induced by a liberation of 5 hydroxytryptamine The diarrhoea is strongly influenced by a 5 HT antagonist parachlorophenylalanine that does not abolish the flush There are however other patterns of hyperemia Some patients develop a bright red colour on the trunk that lasts for some time Others have a continuous cyanotic tinge that is at first regarded as ordinary cyanosis until it is found that it disappears immediately after extirpation of the tumour if this may be radical There are also hyperemic patches in the skin that have been *interpreted as angiomas but are obviously connected* with the tumour

Even if this seems to be a rather wide spectrum of vasomotor processes there is still another very characteristic pattern that seems to indicate the presence of a *gastric* carcinoid tumour We observed a woman with bright red patchy rather long lasting (60) itchy flushing over trunk and extremities This lady produced large amounts of histamine but she had no headaches and was—luckily—achlorhydric A limited number of similar pa has been observed Their biochemical pattern is also typical They may show osteoplastic metastases—otherwise a very unusual finding in this tumour group They have no diarrhoea Without any doubt these symptoms are produced by histamine as Pernow was the first to demonstrate large amounts of this amine in the urine Later several similar cases were described

There exists another condition with histamine overproduction and bright red flushing in a few patients with a disease that the dermatologists call urticaria pigmentosa The lesions may be few a localized to the skin but they may also be wi spread and the disease must then be regarded a mastocytosis Some of these patients liberate la amounts of histamine with generalized flushing l sometimes also with severe hypotension (sho and vomiting of large amounts of gastric jui Their attacks have been provoked in two of c patients by salicylic acid that may obviously b liberator of histamine

In another patient who suffered from hyperte sion but had no other disease we saw intense r flush all over the body twice after taking Meprob It is probable that the flushes seen in anaphyl tic reactions after some drugs but also after b *and wasp stings are caused by the same vasoacti* kinins and/or histamine but why they only appear sensitive persons is an enigma—except in the p tients with mast cell disease

During the very last years considerable inter has been focused on the phenomenon of endocri diarrhoea often but not always accompanied l flushing Medullary thyroid carcinoma (MTC) perhaps the most well observed condition We ha seen a relatively large number of such patients small group among these patients has diarrhoea a a fraction among them show flushes that somewh resemble a carcinoid flush High calcitonin in t serum is a constant finding in widespread MTC a there are patients with very high levels withc diarrhoea or flushing Other neoplastic conditio with diarrhoea are neuroblastoma and sor tumours from the endocrine pancreas Some these cases also show flushing The combinati flush in both skin and intestines seems well wor continued observation

Jan G Waldenström

Acta Med Scand 201 3–7 1977

1α-Hydroxycholecalciferol in the Treatment of Hypoparathyroidism

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BSTRACT In two patients (father and daughter) ith idiopathic hypoparathyroidism one of whom resistant to the action of vitamin D and AT 10 hydroxycholecalciferol (1α (OH)D_3) in doses of 5 µg/day restored the serum calcium concentration the normal range The calcemic effect of 1α D_3 was in both patients due to an increased inal calcium absorption and increased calcium from bone In one of the patients 1α D_3 also increased the renal tubular calcium rption, in the other it did not have this effect, ulting in hypercalciuria as serum calcium rose lack of effect on tubular calcium reabsorption bably accounts for the relative resistance to the tion of 1α (OH)D_3 in this patient compared with other

itamin D has for years been used in the treatment of hypoparathyroidism The normal daily require of *vitamin D is in the order of 0 01 mg (400 IU)* t pharmacological doses are necessary to correct ocalcemia in hypoparathyroidism (1 10) Some atients are resistant to ergocalciferol (vitamin D) ither initially or after some time Some patients y *then respond to cholecalciferol (vitamin D_3) or* o dihydrotachysterol the active compound in AT 10 (6 10) AT 10 is however a mixture of terols with varying biologic activity in different atches (6 10) which makes the treatment with this ompound *unreliable*

The mechanism of the resistance to vitamin D nd D_3 in hypoparathyroidism has been made learer in recent years Vitamin D_3 is metabolized in e liver to 25 hydroxycholecalciferol (25 (OH)D_3) 1 is *further hydroxylized in the kidneys to 1 25* hydroxycholecalciferol (1 25 $(OH)_2D_3$) which is nsidered to be the metabolically active form of amin D (5 7) and 1 hydroxylation of the vitamin is essential for optimal biologic activity Since this process is catalyzed by parathormone (PTH) (8) and depressed by hyperphosphatemia (5) the re sistance to the action of *vitamin D* and D_2 *in hypo*parathyroidism could be anticipated and would be expected to be bypassed by administration of 1 hydroxylated vitamin D compounds

1 25 (OH) D_3 has been synthetized and has been *shown to be active in hypoparathyroid patients re* fractory to other vitamin D compounds (13 16) The synthesis of 1 25 $(OH)_2D_3$ is however compli cated and expensive One of its analogues 1α hy droxycholecalciferol (1α (OH)D_3) first synthetized *by Holick et al (11) and Chalmers et al (4) seems* to possess about the same biologic activity as 1 25 $(OH)_2D_3$ Since this compound is easier and less expensive to synthetize it may be valuable in treatment of several hypocalcemic states

We report the results of treatment with 1α (OH)D_3 in two patients with idiopathic hypopara thyroidism one of whom was resistant to the action of other available vitamin D preparations

METHODS AND PATIENTS

Serum calcium phosphate and creatinine were de termined by Autoanalyzer SMA 12/60 (12) urinary creatinine by the same procedure with another au toanalyzer Phosphate in urine and serum magnesium were measured by atomic absorption spectroscopy Calcium mobilization from bone was evaluated from the calcium/creatinine ratio in fasting morning urine as re commended by Nordin et al (17) The gastrointestinal calcium absorption was measured as described by Berstad et al (3) After a single dose of 10 mCi ^{47}Ca with 100 mg $CaCl_2$ as carrier the percentage absorption of this dose was calculated from measurement of blood concentrations and fecal output of ^{47}Ca The renal tubular reabsorption of calcium was assessed from the excretion of calcium/100 ml glomerular filtrate (Ca_{ex} /100 ml GF) in fasting morn

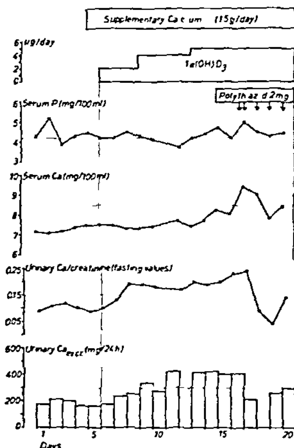


Fig 1 Case 1 Fasting serum calcium and phosphate concentrations calcium/creatinine ratio in fasting morning urine and urinary calcium excretion/24 hours before and during treatment with 1α-(OH)D₃ = lower normal limit for serum calcium upper normal limits for the other parameters

ing urine compared with serum calcium as described by Peacock et al (18)

Case 1 Man born in 1917 Idiopathic hypoparathyroidism was diagnosed in 1972 He was then incapacitated from chorea athetosis dystonia and rigidity Latent tetany was present and radiological examination of the skull showed dense calcifications in the basal ganglia and cerebellum

Serum calcium was 7 1 7 6 mg/100 ml phosphate 4 1–5 8 mg/100 ml and creatinine 0 7–1 3 mg/100 ml Pseudohypoparathyroidism was excluded by a 5-fold increase in urinary phosphate excretion and a more than 20-fold increase in the excretion of cyclic AMP after i v injection 200 USP units PTH

Peroral and i m treatment with vitamin D_2 in doses of ½ mill IU/day were ineffective Correction of a mild hypomagnesemia (serum magnesium 1 2 1 5 mEq/l) did not restore the response to vitamin D On treatment with AT 10 in combination with peroral calcium and polythiazide serum calcium rose AT 10 had however to be given in doses of 10 mg/day and later all available batches of AT 10 were inactive in increasing serum calcium

Case 2 Woman 29 years of age daughter of case 1 family examination showed her to be hypocalcemic with latent tetany Intracerebral calcifications were also present Serum calcium was 6 6–7 3 mg/100 ml phosphate 4 3–5 1 mg/100 ml and creatinine 0 5–0 8 mg/100 ml The diagnosis of idiopathic hypoparathyroidism was confirmed Pseudohypoparathyroidism was excluded by a 3-fold increase in urinary phosphate excretion and an increase in cyclic AMP excretion by a factor of 100 after 2 USP units PTH i v

Treatment was started with 1α-(OH)D_3 (supplied by Løvens Kemiske Fabrik Copenhagen) and peroral calcium

RESULTS

Fig 1 shows that 1α (OH)D_3 did raise serum calcium in case 1 when given in doses of 5 µg/day Concurrently there was an increase in urinary calcium excretion to above 400 mg/24 h The Ca_{excr}/100 ml GF before treatment was high in relation to the low serum calcium (Fig 2) When serum calcium rose upon treatment with 1α (OH)D_3 there was an almost parallel increase in Ca_{excr}/100 ml GF

Addition of polythiazide lowered the urinary calcium excretion although serum calcium was further increased (Fig 1) This effect of the thiazide seemed to be fading when the treatment was discontinued

Fig 1 also shows that the calcium/creatinine ratio in fasting morning urine rose from about 0 10 to 0 18–0 20 The serum phosphate concentration was unchanged Unfortunately the pretreatment intestinal calcium absorption was not measured b

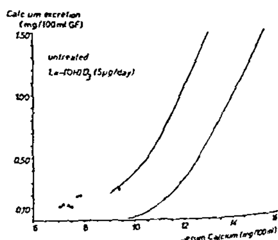


Fig 2 Case 1 Excretion of calcium/100 ml glomerular filtrate in fasting morning urine in relation to the serum calcium concentration before and during treatment with 1α-(OH)D_3 The lines represent mean ±2 S D for normal subjects as defined by Peacock et al (18)

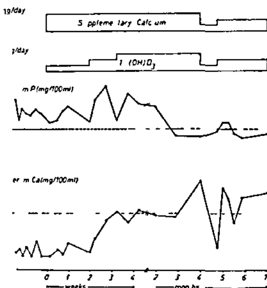


Fig 3 Case 2 Fasting serum calcium and phosphate concentrations before and during long term treatment with 1α-(OH)D_3 = upper normal limit for serum phosphate lower normal limit for serum calcium

When measured twice during treatment with 1α (OH)D_3 the calcium absorption was found to be elevated to 55 and 65% (upper normal limit 50%)

In case 2 1 μg/day of 1α (OH)D_3 had no effect on serum calcium which then rose into the low normal range after a dose increment to 2 μg/day (Fig 3) On 3 μg/day of 1α (OH)D_3 fasting serum calcium rose to 9 9 mg/100 ml and symptoms of hypercalcemia (anorexia and vomiting) developed after meals When the dose of 1α (OH)D_3 was lowered to 2 μg/day serum calcium fell to the pretreatment level but rose as soon as the dose was adjusted to 2 μg/day (Fig 3)

In case 2 also the Ca_{excr}/100 ml GF before treatment was increased in relation to the hypocalcemia When serum calcium rose during treatment with 1α (OH)D_3 the urinary calcium excretion rose from 0 to 280 mg/24 h The Ca_{excr}/100 ml GF also rose however in relation to serum calcium (Fig 4) it in fact decreased

Her intestinal calcium absorption before treatment was within the normal range (39%) and rose on 1α (OH)D_3 to 73% The calcium/creatinine ratio in fasting morning urine rose from 0 05–0 10 to 0 17–0 24 During long term administration of 1α (OH)D_3 there was no increase in serum phosphate concentration which instead fell back into the high normal range

Serum alkaline phosphatase SGOT SGPT bilirubin and normotest were controlled regularly and all values remained within the normal range during the treatment

DISCUSSION

In two related patients with idiopathic hypoparathyroidism serum calcium increased upon treatment with 1α (OH)D_3 In patient 1 who was resistant to the action of vitamin D and available preparations of AT 10 a dose of 5 μg/day was necessary to increase serum calcium while patient 2 was normocalcemic on 2 μg/day In the latter patient 1 μg/day was not sufficient to elevate serum calcium and 3 μg/day caused symptoms of postprandial hypercalcemia presumably caused by increased intestinal calcium absorption

Measured by isotope technique 1α (OH)D_3 was in both patients found to increase the intestinal calcium absorption The normal calcium absorption found unexpectedly before treatment in case 2 is difficult to explain Synthesis of another metabolite with specific effect on intestinal calcium absorption (24 25 (OH) D_3) has been reported to occur when the synthesis of 1 25 (OH) D_3 as in hypoparathyroidism is shut off (5) and this might possibly account for the observation

According to Nordin et al (17) the fasting calcium/creatinine ratio in urine is independent of intestinal calcium absorption and reflects the bone resorption Since the fasting urinary cal

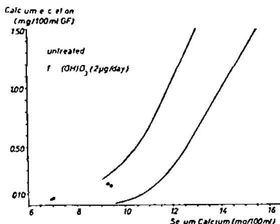


Fig 4 Case 2 Excretion of calcium/100 ml glomerular filtrate in fasting morning urine in relation to the serum calcium concentration before and during treatment with 1α (OH)D_3 The lines represent mean±2 S D of subjects as defined by Peacock et al (18)

cium/creatinine ratio rose from 0 05–0 10 to 0 17–0 24 in both patients during treatment with 1α (OH)D_3 the serum calcium enhancing effect of the vitamin D analogue was probably also due to increased calcium mobilization from bone

Vitamin D might also affect serum calcium by an effect on the renal tubular reabsorption of calcium The tubular calcium reabsorption before treatment was low as estimated from Ca_{excr}/100 ml GF related to serum calcium concentration During treatment with 1α (OH)D_3 the increased bone calcium mobilization and intestinal calcium absorption resulted in a rise in the glomerular filtration of calcium and the calcium excretion/24 h rose in both patients The Ca_{excr}/100 ml GF when related to serum calcium did however fall in case 2 indicating an increase in tubular reabsorption of calcium In case 1 no effect of 1α (OH)D_3 on tubular reabsorption of calcium was observed resulting in a renal leak of calcium with the risk or urinary concrements and nephrocalcinosis The lack of effect on tubular calcium reabsorption probably accounts for the relative resistance to the action of 1α (OH)D_3 in this patient in whom 5 μg/day of the compound was necessary to raise serum calcium compared with 2 μg/day in case 2

Polythiazides have been shown to increase the [illegible]lar reabsorption of calcium (15) and when add[illegible] to 1α (OH)D_3 serum calcium rose and urinary calcium decreased in case 1 The thiazides were however given for only a few days and no definite conclusions can be drawn concerning their value in long term treatment of vitamin D resistant hypoparathyroidism

Previous studies in hypoparathyroid men (16 21) and in thyroparathyroidectomized rats (19) have shown that 1 25 (OH)$_2D_3$ and 1α (OH)D_3 increase the intestinal calcium absorption and this is the most constant effect of the vitamin and its analogue on the calcium metabolism The effect on intestinal calcium transport is independent of PTH while the effect on bone resorption (9 22) and possibly the effect on renal tubular calcium transport may be dependent on the presence of PTH to be optimal Therefore the effects of 1α (OH)D_3 in hypoparathyroidism could be quantitatively and qualitatively different from those in patients with intact parathyroid function

In studies of the bone resorption as evaluated from the urinary hydroxyprolin excretion Neer et al (16) found no effect of 1α (OH)D_3 on bone resorption in hypoparathyroid patients In hypo[illegible]thyroid rats however 1α-(OH)D_3 increased ur[illegible] hydroxyprolin excretion (19) and the ris[illegible] calcium/creatinine ratio in fasting morning urin[illegible] ported by Russel et al (21) is in agreement wit[illegible] observations and indicates that 1α-(OH)D_3 ma[illegible] crease the calcium mobilization from bone in h[illegible] parathyroid patients

Conflicting results are also reported on the e[illegible] of 1,25-(OH)$_2D_3$ and 1α-(OH)D_3 on the tu[illegible] calcium reabsorption in hypoparathyroidism [illegible] 20 21) Our observations agree with those of R[illegible] et al (21) and indicate that 1α (OH)D_3 may incr[illegible] the calcium reabsorption at least in some pat[illegible] Due to the variable effect of 1α-(OH)D_3 on tubular calcium handling in hypoparathyroid estimates of the urinary calcium excretion are [illegible] liable as an indicator of the serum calcium con[illegible]tration

In rachitic rats 1,25-(OH)$_2D_3$ and 1α-(O[illegible] stimulate the intestinal phosphate absorption increase the serum phosphate concentration The effect of vitamin D on renal tubular phos[illegible] handling is controversial (19) In thyroparathy[illegible] ectomized dogs (20) and rats (19) however [illegible] (OH)D_3 has been reported to increase the t[illegible]lar phosphate reabsorption and raise the se[illegible] phosphate concentration

In hypoparathyroid patients a further ris[illegible] serum phosphate is undesirable as it increase[illegible] risk of deposition of intracerebral calcium-p[illegible]phate complexes However the serum phosp[illegible] concentration did not rise in either our or othe[illegible] ported patients with hypoparathyroidism but either unchanged or even fell during treat[illegible] with 1α (OH)D_3 (2 16 21) In the cases repo[illegible] by Neer et al (16) the fall in serum phosphate accompanied by increased urinary phosphate [illegible]cretion In addition to a direct effect of vitamin [illegible] tubular phosphate handling the vitamin may affect the renal handling indirectly by normali[illegible] serum calcium wich per se has a direct phosphat[illegible] effect (14)

1 25 (OH)D_3 and 1α-(OH)D_3 are found to be [illegible] times more active than vitamin D_3 in resto[illegible] serum calcium concentration in vitamin D defic[illegible] animals and men (16) In contrast to the re[illegible] in parathyroid intact animals and men the [illegible]droxylated compounds are 100–500 fold m[illegible] active than vitamin D_3 in patients with hypop[illegible]thyroidism (16)

The high potency of 1α $(OH)D_3$ involves the risk of hypercalcemia developing even after small dose increments The rapid cessation of the compound s action when treatment is discontinued is therefore a great clinical advantage This makes it possible to correct hypercalcemia and adjust the dosage in a few days (16 21) compared with weeks or months after treatment with vitamin D_3 1α $(OH)D_3$ may be the drug of choice in treatment of hypoparathyroidism and other conditions of vitamin D resistance due to defective 1 hydroxylation of the vitamin D

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Energy Deprivation in Man—Methodological Problems and Possibilities

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BSTRACT Various approaches to the study of hysiological and behavioural effects of energy de rivation are presented and discussed An experi iental, total energy deprivation in 20 healthy human olunteers during an 11-day period, preceded and llowed by 4 day control periods, is described otentially pathogenic reactions which occurred in pite of precautions are analysed It is concluded iat the present controlled real life model with acute tal energy deprivation serves the purpose of study ig hypotheses concerning potentially pathogenic iechanisms occurring in deprivational situations nder conditions of natural and man made cata trophes

ne of the most prevalent medical and psycho- ocial problems of our time is the chronic food eprivation facing hundreds of millions of indi iduals all over the world It is well known that uch exposure—even when it is not lethal—does ause marked medical and psychological disabilities) 11 20) A related problem concerns the effects f food deprivation on man during natural as well s man made catastrophes for instance war Both pes of circumstance may leave large populations tally or almost totally deprived of food Although rgently desired it is usually considered un easible in the foreseeable future to eliminate the orld food shortage that leads to noxious effects 12)

Disability and death from malnutrition are known) occur before the human body s energy reserves ave been exhausted (10) It follows that mechanisms other than the depletion of energy sources are of pathogenic significance one is considered to be an increased susceptibility to infectious agents Hence it is important to clarify the patho genic *mechanisms* with a view to establishing thera peutic and/or prophylactic intervention Theoreti cally this approach can involve interventions in endocrine metabolic and immune processes and would complement attempts to increase the avail ability of food

Survival is also dependent on motivational and behavioural aspects of reactions to starvation such as starvation induced apathy (14 17) When such reactions reach critical levels people may continue to starve even though a minor effort would give access to life saving food By interfering also with such potentially pathogenic mechanisms disability and death might theoretically be postponed Various strategies can be adopted to study the research implications of these approaches

Field studies in developing countries

The strategy closest at hand is to study malnourished populations in developing countries Such studies have been reported by many authors (4) Investigations of this type seem to have an obvious face value particularly with regard to the effects of long term starvation However exposed populations are far from homogeneous with re gard to their type of malnutrition (e g marasmus kwashi orkor) a fact that is often overlooked as pointed out by Avila et al (2) Even when homogeneous in this respect exposed populations face environmental ing not only starvation but a very compl pattern of noxious influences such climatic extremes and severely

economic cond tions Furthermore cognitive and emotional reactions evoked by severe starvat on and in particular by the prospect of impending death may have physiological concomitants All these circumstances can influence the processes under study (18)

A second strategy is to study reactions to the adm nis tration of food to ind viduals previously exposed to pro longed starvat on But th s is commonly done in connec tion with hospitalization whereupon other environ mental factors are changed in add tion to the d etary regime (1 7 21) These "extraneous changes rarely measured and controlled m ght well have profound effects direct or ind rect on the variables under study With these research strategies it is hardly possible to partial out the importance of energy deprivation from the very complex environmental influences

Starvat on in pat ents

Research in patients on the effects of starvation has focused on two extreme groups namely anorexia nervosa and obes ty Although malnutrition is a prom nent feature of anorexia nervosa the relevance of th s condition as a model in the study of energy deprivation is l mited by psychological and phys ological deviations in these pa tients (19 22) such as the occurrence of malabsorption and self-induced vom ting

It m ght be argued that therapeutic starvation in obes ty presents an eth cally acceptable and even therapeutically mot vated model for energy deprivation (5 15 23) But results from such studies are not always d rectly applicable to normal weight persons partly because obesity may be accompanied by metabol c changes (3) d the obese person under food deprivat on is ut l zing s own abundant energy reserves and partly because the attitudes of obese subjects towards food and food depriva tion clearly differ from those in normal populat ons (6 13)

Starvation in healthy volunteers (fast ng)

An alternative approach would be to study the effects of energy deprivation in healthy normal we ght sub jects who period cally refra n from food because this is assumed to be benef cial to health They are usually adherents of health movements and will ngly volunteer for such exposure However their h gh mot vation for fasting compl cates a comparison with subjects whose attitude to food and its deprivat on is more normal They have very spec f c preconcept ons of how they should react and often know how they d d indeed react during earlier fasting experiences wh ch introduces a bias to their self-reports and poss bly through psychophys o-logical relationships influences at least some phys o logical variables as well Finally the r regimes often include various fruit ju ces and herbs

A further poss bility is to study normal healthy volun teers without previous fast ng experience and without f rm preconceptions about the benef t pleasure or distress of food deprivation Such stud es have been performed usually in metabol c wards (8) Methodological objec tions concern the quest onable normal ty of individuals volunteering in such stud es as well as the h ghly arti f cial environmental conditions in which such stud es are performed (14)

SPECIAL PROBLEMS AND APPROACHES TO THEIR SOLUTION

We feel that the research strategy described below sa fies the requ rements ra sed at all events by s tuati like natural or man made catastrophes expos ng la populations to total relatively transient starvation results may also serve as a gu de to hypotheses ab pathogenic mechanisms in chron c starvat on T research strategy was applied in a study carried out 1974 the results of which will be published separat It was concerned ma nly with immune endocrine and havioural reactions The methodolog cal aspect of study has been described in detail elsewhere (16)

Select on of subjects

Our subjects were drawn from a populat on wh ch actua runs a risk of being exposed to man made catastroph namely sold ers and off cers A batall on was given ver informat on about the proposed study After exclus on volunteers who were smokers on a drug or d et regimen ill or markedly over or under we ght remainder was given deta led written info mat on The who accepted partic pation formed the population und study Details of the procedure are given elsewhere (1

The 20 subjects all males aged 25±2 years (me ±S E M) passed a standard med cal check-up Fourte formed the starvation group and six were ass gned to control group The ass gnment was done in such a w that the two groups had the same he ght/we ght ratio T assignment was not reported to the subjects until t evening before the start of starvation to equal ze expect t ons and ant c pat on during the pre starvation contr period

Design of the study and proced res

The study lasted 19 days subd v ded into two 4-d control periods preced ng and follo ving an 11-day starv t on period for the starvat on group The control grou was exposed to the same procedures except the 11 days food deprivat on During the four pre starvat on days 20 subjects were on a standard zed d et Details of the d are given elsewhere (16)

During starvation days 1–11 the starvation group w deprived of all food whereas the control group remaine on the standard zed d et Water was available ad l b th fasting subjects being instructed to ingest at lea t 3 l dai Sod um bicarbonate and potass um chloride were su st tuted orally on an ind vidual basis to avoid electroly and acid–base balance d sturbances the dosage be ng ca culated from serum analyses On the even ng of the 11t starvation day the food deprivat on was term nated an on the following days there was a gradual return to th standard d et On the last two days of the post starva t on period all subjects had returned to th s diet Through out the study no drugs or caffeine or alcohol-contain ing beverages were allowed

Throughout the study all subjects took part n routin mil tary train ng but not in athlet cs or any other strenuou physical exercises The rout nes were made equ alen

from day to day and details of them have been given elsewhere (16)

Venous blood samples were drawn as indicated in Fig 1 always around 8 00 a m after at least an overnight fast Immunizations were performed on starvation days 1 and 10 in half of the starving group on each occasion and in the control group on the first Skin tests for measuring delayed hypersensitivity were carried out on starvation day 10 Urine samples were taken thrice daily at 6 00–7 30 p m 7 30–9 00 p m and after the hours of sleep ECGs were recorded on the first pre starvation day on the fourth seventh and tenth starvation days and on the second post starvation day always in the afternoon

Ethical considerations and medical safety measures

Exposure of semi starving populations to acute experimental starvation was considered out of the question for ethical reasons Even the exposure of healthy volunteers to 11 days of starvation can of course be questioned from the ethical point of view To safeguard our subjects and to comply with all the relevant rules in the Helsinki declaration we used the following approach

(*a*) All subjects were given detailed written and verbal information concerning the objectives routines and potential risks (*b*) The subjects were allowed to withdraw from the study at any time and without giving any explanation or motives (*c*) The study was unanimously approved by the Ethical Committee of the Karolinska Institute and by a permanent representative council of the population from which our subjects were drawn (*d*) No pressure whatsoever was allowed to be exerted on the group from which the experimental subjects were drawn either by their superior officers their peers or any other groups or individuals (*e*) The subjects were remunerated for their participation in view of the fact that almost all their leisure time was spent on experimental routines However the remuneration was relatively small (US $2 — per hour of effective leisure time) so as not to seduce potential candidates into taking part primarily for economic reasons or tempt them not to withdraw from the study even if they felt the need to do so (*f*) Medical supervision throughout the study was frequent and careful

RESULTS

Clinical phenomena observed during and after the course of starvation

The mean weight loss in the starvation group was highly significant 6 4±0 3 kg and most pronounced in the early part of the starvation period probably mostly due to water loss (Fig 2) The control group showed no significant changes in weight

Ketonuria reflecting metabolic adaptation to the energy deprivation occurred within two days and persisted throughout the starvation period except

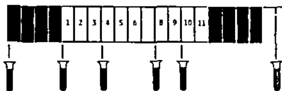


Fig 1 The course of the experiment Venous blood samples indicated by test tubes were obtained around 8 00 a m Black fields indicate pre and post starvation days

in one subject whose ketonuria disappeared on one occasion following consumption of some chocolate Accordingly our approach turned out to be effective in accomplishing the starvation aimed at

In two subjects starvation was discontinued on the 8th starvation day In one of them serum creatinine rose from 1 0 to 2 1 mg/100 ml blood most probably reflecting a moderate dehydration It returned to normal pre starvation values a few days later In the other subject ECG discovered generalized negativity in the T wave not present four days earlier No clinical symptoms or elevations of serum ALAT ASAT and LDH were reported or observed at any time The ECG pattern remained essentially unchanged for several months and then slowly returned to normal

On the evening of the 10th starvation day the starvation was discontinued in another two subjects One could not ingest fluid in sufficient amounts eventually leading to dehydration with increases in Hb haematocrit and serum albumin His conditions returned to normal within a few days In the other subject increases in serum uric acid and suspected haematuria were noted these normalized soon after the return to a normal diet None of these reactions in the four subjects were accompanied by any clinical symptoms Starvation was always discontinued at the request of the experimenters and all four subjects remained in the experiment with regard to all routines except starvation

Three of the starving and two of the control subjects exhibited mild symptoms of an upper respiratory tract infection during the second half of the starvation period No complications occurred and restitution was complete within a few days All subjects were followed up two days after the end of the post starvation period No additional infections other disorders late complications or complaints were reported

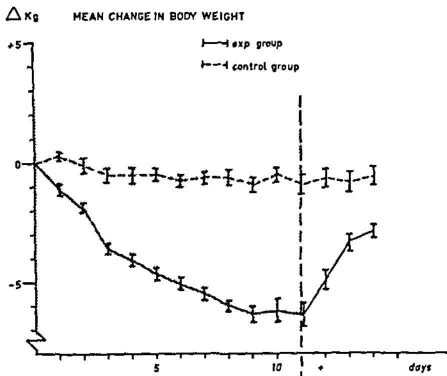


Fig 2 Change in body weight (mean and S E M) for the experimental and control groups Shaded area = the starvation period

CONCLUSIONS

As already pointed out results with regard to the variables under study will be presented in another text The present paper is concerned exclusively with methodological matters With regard to the latter our conclusions read as follows

1) The co operation of healthy human volunteers can be secured if detailed information is given and repeated about the motives for the study its design and procedures 2) The ethical requirements can be fulfilled by strict adherence to the Helsinki declaration 3) Subjects can be kept under relatively strict control conditions in spite of participating in what are for them real life activities 4) Potentially pathogenic reactions may occur occasionally even though extensive precautions are taken (This implies some criticism of self administered or otherwise loosely controlled radical weight reducing regimens) 5) Expectations and anticipation can be controlled if information concerning group assignment is withheld until just before the start of the starvation period 6) A control group makes it possible to partial out effects created by the experimental situation *per se* whatever the dietary regimen 7) The post starvation control period serves to demonstrate effects of discontinuing starvation 8) Circadian rhythm in physiological and psychological functions must be taken into account in the timing of activities and measurements

ACKNOWLEDGEMENTS

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Effects of Total Energy Withdrawal (Fasting) on the Levels of Growth Hormone, Thyrotropin, Cortisol, Adrenaline, Noradrenaline, T_4, T_3 and rT_3 in Healthy Males

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ABSTRACT Ten days of total energy deprivation evoked the following endocrine changes in 12 healthy normal weight males early and marked reductions and increments in the blood levels of T_3 and reverse T_3 respectively, with rapid returns to pre starvation levels after refeeding a slight and late decrease in the blood levels of T_4 a minute reduction of the blood levels of TSH a pronounced increase in the blood levels of growth hormone but a return towards pre exposure levels even before discontinuation of starving a minor and gradual enhancement of the blood levels of cortisol and an increase in nocturnal urinary adrenaline excretion It is assumed that these changes reflect a complex regulatory mechanism the purpose of which is to secure adequate energy supply to vital organs

Food deprivation exerts one of today s most prevalent environmental influences on mankind Disability and death from malnutrition usually occur before the energy reserves in the human body are exhausted (9) It follows that mechanisms secondary to starvation are likely to have pathogenic significance Therefore a study was initiated to evaluate the endocrine immunologic and psychologic effects of complete energy withdrawal in healthy non-obese males

Most studies concerning endocrine effects of energy deprivation have been carried out in subjects suffering from malnutrition or obesity (6 16 20 27 29 30) In malnourished subjects noxious influences such as infections may affect the variables under study Moreover cognitive and emotional reactions evoked by exposure to starvation can lead to endocrine manifestations in healthy as well as diseased individuals (17 18) In addition results obtained from studies on obese subjects are not always applicable to normal weight persons (3) In an attempt to control such influences the present study was carried out under standardized conditions on healthy non-obese volunteers and also included a control group of non starving subjects Both groups maintained normal physical activity

A detailed technical description and the results of the immunologic and psychologic parts of the study will be published separately This paper presents the endocrine part

MATERIAL AND METHODS

Subjects

Twenty healthy males army officers and soldiers with an average weight of 77 2±2 5 kg (mean and S E M) and age of 25±2 years (mean and S E M) volunteered (informed consent) to participate in the study which was carried out in 1974 after approval by the Ethical Committee of the Karolinska Institute Stockholm and by the permanent representative council of the military personnel concerned

Experimental procedure

After 4 pre starvation days with standardized ordinary food ad lib 14 subjects were assigned to an

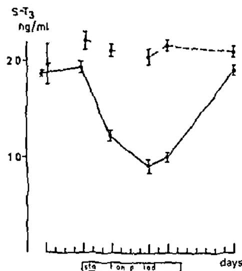


Fig 1 Serum T_3 levels (mean ±S E M) ●—●=starving sui ects O O=controls

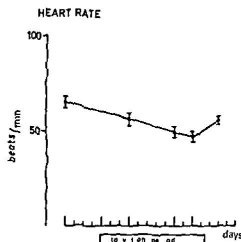


Fig 2 Heart rate in the starving subjects ±S E M)

(starving) group and 6 to a control group with equalization of the groups with regard to the height/weight ratio The control group continued on the standard food regimen throughout the study The experimental group was deprived of all food and allowed to drink only non calorie beverages for the following 11 starvation days

All subjects were kept under strict medical supervision Individual serum electrolytes were measured regularly and the fasting subjects were given sodium bicarbonate and potassium chloride accordingly In an attempt to avoid dehydration the daily intake of fluid was not allowed to be less than 3 l After the 11 days of complete starvation during which the subjects exhibited a mean ght loss of 6 4±0 3 kg (mean and S E M) (range 8 5–4 kg) standard food was gradually reintroduced during the last four days (post starvation period) Two subjects discontinued the starvation on the eighth starvation day at the request of the researchers and their values are excluded giving $n=12$ in the starvation group No caffeine-containing or alcoholic beverages were allowed during the study Throughout the study all subjects took part in routine military training but not in athletics or any other strenuous physical exercises The routines were kept similar from day to day throughout the study

Blood and urine sampling

Blood samples were drawn for hormone analyses at 8 00 a m on pre starvation day 1 on starvation days 1 4 8 and 10 and on the sixth post starvation day Further details are given elsewhere (17)

Urine samples for the measurements of catecholamines in this part of the study were collected during each night s sleeping period Data are given as the sum of each of the following periods the three last pre starvation nights the four first and four middle and three last starvation nights and the four post starvation nights

Hormone analyses on serum and plasma

Serum and plasma were prepared from the blood sa and frozen at −20°C until analysed fo hormonal con The concentrations of 3,5,3 triiodothyronine (T_3) roxine (T_4) 3 3 5 triiodothyronin (reverse T_3) thyrotropin (TSH) and growth hormone (GH) measured by radioimmunoassays (4 5 23 24 31) cortisol concentrations were determined by a fluoror technique (8) The normal values (±2 S D) for T_4 T_3 and rT_3 for the age group under study are 39 1 65±0 46 and 0 45±0 2 ng/ml Plasma TSH refe values are >10 mU/l (mean 3) For serum cortis normal value at 8 00 a m is 13 6±5 µg/100 ml S GH values below 3 ng/ml are considered normal

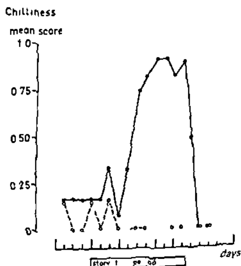


Fig 3 Mean score of chilliness Symbols as in Fig 1

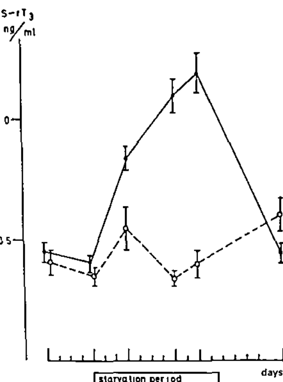


Fig 4 Serum rT_3 levels (mean ±S E M) Symbols as in Fig 1

Catecholamine concentrations in urine

Freshly voided urine was acidified to pH 3 and frozen at −70°C until analysed for catecholamine contents with an AutoAnalyzer fluorescence method (?)

Miscellaneous

Subjects were asked to rate their perceived chilliness daily at 7 00 a m using a 10 cm anchored graphic rating scale ranging from not at all to much The recorded ratings were divided into the following scores 0 (no chilliness 0–5 mm) 1 (slight chilliness 6–33 mm) 2 (medium chilliness 34–66 mm) 3 (much chilliness 67–100 mm) Heart rate was determined by ECG recordings as indicated in Fig 3

Statistical procedures

The Mann Whitney test was used for evaluation of changes between the groups and the Wilcoxon s signed rank test for evaluation of changes for both groups over time As the level of significance $p<0.01$ was chosen

RESULTS

Serum T_3, pulse rate and chilliness

During the pre starvation period i e in samples obtained 4 days before and at the beginning of the starvation the serum T_3 levels of both groups remained unchanged Within three days of energy withdrawal the T_3 levels were reduced and a further reduction occurred within another four days (Fig 1) These reductions were statistically significant compared both with the pre starvation days and the concomitant control group values The T_3 levels remained low until refeeding (Fig 1) During starvation these levels fell from initial mean values near the upper limit of the normal range to values approaching the hypothyroid range In the control subjects the T_3 levels remained essentially unaltered throughout the experiment

The reduction of T_3 was accompanied by a statistically significant decrease in heart rate (Fig 2) and an increase in feeling of chilliness (Fig 3)

Serum reverse T_3 (rT_3)

Like the T_3 levels those of rT_3 remained essentially unchanged during the pre starvation period in both groups During starvation however the levels of rT_3 followed a pattern opposite that of T_3 in the starvation group within three days of fasting there was a significant increase and a further increment within the next four days was significantly different from the mean value of the control group (Fig 4) The starvation induced increase was more than 2 fold the values exceeding the upper normal limit

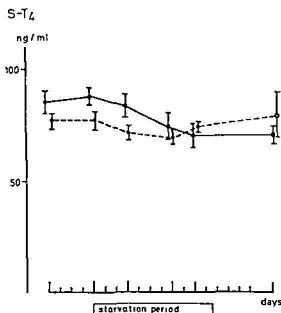


Fig 5 Serum T_4 levels (mean ±S E M) Symbols as in Fig 1

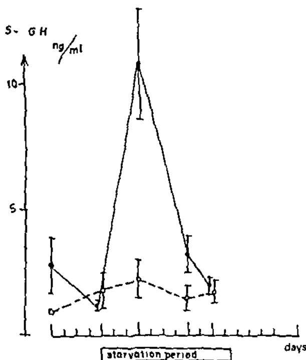


Fig 6 Serum GH levels (mean ±S E M) Symbols as in Fig 1

After refeeding the rT_3 levels declined rapidly to pre starvation levels No significant change oc curred in the control subjects

Serum T_4

During the pre starvation period the serum T_4 levels remained virtually unchanged in both groups and remained so in the control group throughout the study (Fig 5) During starvation there was a minor gradual decrease The mean value on starva tion day 10 was significantly lower than that on starvation day 1

TSH in plasma

In some individuals the blood sample volume did not suffice for analysis of the TSH content at each interval Consequently the statistical significance of differences was calculated only from the data of days 1 and 10 when $n=12$ The plasma TSH levels displayed a significant but minute numerical de crease in the starvation group from 4 0±0 2 mU/l (mean ±S E M) on starvation day 1 to 3 5±0 1 mU/l on starvation day 10 Corresponding values for the control group are 4 6±0 4 and 4 2±0 1 On day 10 the mean difference between the groups was not significant

Serum GH

During the pre starvation period the level serum GH in both groups were close to the l limit of measurement and displayed a marked significant increment within three days of starv (Fig 6) A return to pre exposure values occu even before discontinuation of fasting There no significant changes in the serum GH levels in controls

Serum cortisol

During the pre starvation period there were significant changes in the blood levels of cor in either group (Fig 7) and in the control g they remained unchanged throughout the st During starvation the levels were enhanced only gradually and to a minor degree, the differ reaching significance on starvation day 10 starvation day 1 and control group experime day 10)

Urinary catecholamines

During the pre starvation period there were significant differences between the groups regard to adrenaline and noradrenaline level urine During the middle and last thirds of starvation period increased urinary levels adrenaline were found (Fig 8) the values b significantly higher than those in the control g for corresponding periods and those in the sta tion group before and after starvation The uri levels of adrenaline were significantly lower than before starvation

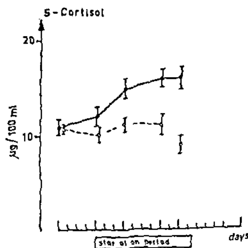


Fig 7 Serum cortisol levels (mean ±S E M) Symbol in Fig 1

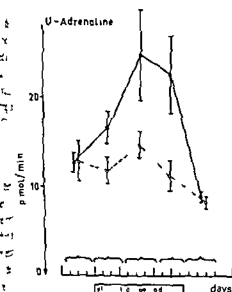


Fig 8 Urinary levels of adrenaline (mean ±S E M) Symbols as in Fig 1

During starvation the numerical mean values of urinary noradrenaline were gradually reduced but the difference was not significant (Fig 9) The control group showed no significant changes in adrenaline and noradrenaline levels in urine during the study (Figs 8 and 9)

DISCUSSION

In the present study, a number of endocrine reactions have been recorded in response to total energy deprivation (fasting) Thus the serum T_3 levels were pronouncedly reduced whereas those of T_4 were slightly diminished and those of TSH showed a very minute reduction The serum rT_3 levels on the other hand were markedly increased and there was also a transient increase in the serum GH levels and a minor and gradual increment of the serum levels of cortisol In addition there was a rise in urinary adrenaline levels

A decrease in serum T_3 has been observed previously in obese subjects undergoing voluntary starvation (27) in patients with anorexia nervosa (21 22) and in subjects with malnutrition (6 15) In the present study no essential change in the T_3 levels occurred during the pre starvation period in either group or during the whole experimental period in the control group It therefore seems to have been established that energy withdrawal as such induces a rapid and pronounced reduction of the blood levels of T_3

Vagenakis et al (32) reported recently that the starvation induced reduction of the serum T_3 levels is associated with a concomitant increase in the rT_3 levels This finding is confirmed by the present study Investigations by our group (33) have shown that rT_3 like T_3 is both a normal secretory product of the human thyroid and an extrathyroidal metabolite of T_4 Accordingly the altered rT_3/T_3 proportion in fasting subjects could be due either to changes in the secretion of the two iodothyronines or to alterations in the relative proportions of T_4 being deiodinated to T_3 and rT_3 or to changes in the clearance of the iodothyronines The reduction of the plasma TSH level though minute could possibly reflect an inhibition of TSH secretion which would be followed by a reduced iodothyronine secretion from the thyroid However as the secretion of not only T_3 and T_4 but also rT_3 depends on TSH (33) the increase in rT_3 can hardly be secondary to a reduced secretion of TSH Hence it is more likely that the opposed changes in the T_3 and rT_3 levels result from an alteration in the proportions of extrathyroidal T_4 being deiodinated to T_3 and rT_3 Irrespective of whether the starvation induced change in the rT_3/T_3 ratio is secondary to intra or extrathyroidal reactions the fact that starving subjects produce more of the metabolically inactive rT_3 and less of the active T_3

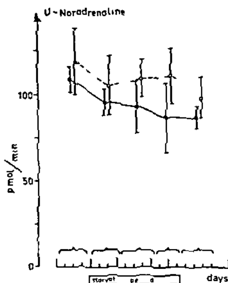


Fig 9 Urinary levels of noradrenaline (mean ±S E M) Symbols as in Fig 1

appears to be a biologically adequate adaptation to the withdrawal of energy supply, as pointed out by Vagenakis et al (32)

During starvation the serum T_4 levels displayed a small and gradual decrease although well within the normal T_4 range Most previous investigations have recorded either unchanged or reduced T_4 levels in starving subjects (6 27 32) The T_4 decrease though small may signify that the secretory activity of the thyroid is reduced by starvation but the T_4 levels in serum may also be reduced by extrathyroidal mechanisms starvation appears to reduce the T_4 binding capacity of serum proteins (15) and different investigators have indeed recorded an increase in the fraction of unbound T_4 (6 27 32)

Corticosteroids have recently been found to reduce T_3 and enhance rT_3 production (7) Hence it could be argued that the changes in the T_3 and rT_3 levels recorded in the present study were secondary to a starvation induced increase in the blood cortisol levels Such an increment was indeed recorded in the subjects of this study However this is not a likely explanation since the increment of blood cortisol was not only very small but also occurred later than the decrease in T_3 Instead as it is known that cortisol levels may be raised in hypothyroid subjects due to a decreased metabolism of the steroid (29) it is quite possible that the recorded increment of the cortisol levels was secondary to the reduction of the T_3 levels Indeed the relatively small and late increment of the cortisol levels in serum casts doubt on the assumption that starvation is a potent stimulant of adrenocortical activity Rather the present findings indicate that the pronounced increments of the cortisol levels recorded in malnourished subjects (1) are provoked by factors concomitant to malnutrition e g infectious diseases (25)

Another endocrine response to starvation was the marked rise in the serum concentrations of GH occurring within three days of fasting This agrees with observations by others (10 19 26) The absence of such GH changes in the control group as well as in the fasting subjects during the pre starvation period indicates that the increments of GH were evoked by starvation as such

In contrast to the findings with the iodothyronines it was observed that the GH levels returned towards normal well before discontinuation of fasting It cannot be excluded that the increment of GH is due to an inhibition of the metabolism or clearance of the hormone but an enha[illegible] secretion would seem to be a more likely exp[illegible] tion Irrespective of which explanation is corre[illegible] is of interest to note that the serum levels of [illegible] were increased and of T_3 reduced Both cli[illegible] and experimental observations infer a functi[illegible] interrelationship between GH and thyroid [illegible]mone(s) Thus GH secretion is reduced in hy[illegible]thyroidism and it is restored to normal whe[illegible] patient becomes euthyroid (11) Moreover, [illegible] increment of GH in response to induced hyp[illegible]cemia is smaller in hyperthyroid than in euth[illegible] subjects (34) In other words excess of th[illegible] hormone(s) appears to diminish GH secretion [illegible]cordingly the present observations could be in[illegible]preted to mean that reduction of the T_3 le[illegible] facilitates GH secretion An additional possib[illegible] is that an increase in rT_3 stimulates the secre[illegible] of GH On the other hand in either situation [illegible] would expect the GH levels to remain enha[illegible] as long as the T_3 and the rT_3 levels were redu[illegible] and enhanced respectively which was not [illegible] case Alternatively the increment of GH could [illegible] causal factor of the altered levels of T_3 and rT_3

Judging from the amounts of catecholamine[illegible] nocturnal urine the excretion of adrenaline [illegible] enhanced whereas that of noradrenaline was [illegible] altered Changes in the urinary levels of catec[illegible]amines can be secondary to altered rates of [illegible]thesis release turnover and excretion and to [illegible] combination of them Thus interpretations mus[illegible] purely speculative If one assumes that incre[illegible] levels of excretion reflect an enhanced sympa[illegible] adrenomedullary activity the findings infer that adrenomedullary release of adrenaline was [illegible]hanced during starvation and reduced on [illegible] feeding (13 28) In this context it is of interes[illegible] note that the decrease in the T_3 and T_4 levels [illegible] have resulted from an increased adrenomedull[illegible] activity since adrenaline has been found [illegible] enhance the peripheral deiodination of T_4 and [illegible] at least in certain animals (12 14) On the ot[illegible] hand the catecholamine changes may have b[illegible] both secondary to and causes of the altered io[illegible]thyronine levels

In conclusion it is apparent that acute t[illegible] energy withdrawal induces a number of endocr[illegible] manifestations which seem to be biologically a[illegible]quate e g to maintain sufficient glucose levels [illegible] normal brain function by mobilizing glucose [illegible]

arious sources and to reduce the metabol c rate n per pheral tissues The causal interrelations of hese endocrine changes require further explora on

ACKNOWLEDGEMENTS

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Calcium, Phosphate and Albumin in Serum

A Population Study with Special Reference to Renal Stone Formers and the Prevalence of Hyperparathyroidism in Middle aged Men

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ABSTRACT Serum values for calcium phosphate and albumin have been determined in a population study of 2322 49–50 year-old men participating in a health examination survey Calcium and albumin were significantly correlated ($r=0.34$) but adjustment for albumin only caused minor effects on the distribution of calcium No inverse relationship was found between calcium and phosphate Seasonal variations over the three years of the health survey could not be established for either calcium or phosphate whereas there was a slight tendency for albumin to decline during summer The prevalence of hyperparathyroidism (HPT) in this population of men up to the age of 50 was 0.3% and among those with recurrent renal stones 5 3% All subjects with verified HPT had a history of recurrent renal stones One man on thiazide treatment had a slight elevation of calcium which returned to normal after cessation of the drug No other case of hypercalcemia besides those caused by HPT was found Mean values and frequency distributions for calcium, phosphate and albumin were almost identical in renal stone formers and matched controls Hence it seems likely that other factors than those which markedly affect serum levels of calcium and phosphate are of major importance in common renal stone formation

High serum values for calcium are not unusual in surveys of hospitalized or ambulatory patients and prevalence figures up to 2 2–2 9% have been reported (6 38) Only a few studies have been concerned with the prevalence of hypercalcemia in the population but it seems that even among apparently healthy individuals high serum calcium values are not infrequently detected (8 40) Hypercalcemia of any cause may produce hypercalciuria and renal stones Hence determinations of serum calcium are often performed in patients with stone disease The commonest cause of hypercalcemia in such patients is hyperparathyroidism (HPT) Previous studies have however dealt with patients attending hospital care and the prevalence of HPT among unselected stone formers is not known

In this study we present the results of determinations of serum calcium phosphate and albumin in a population study of middle aged men and calculate the prevalence of HPT in this population as well as among its renal stone formers The investigation was also concerned with the question whether stone formers as a group displayed any disturbances in serum calcium and phosphate compared with the healthy population

MATERIAL

A health examination survey was offered all men living in the City of Uppsala and born in 1920-24 From Sept 1970 to Sept 1973 2322 men were examined giving a participation rate of 83 9% (15) From this survey the following groups were selected for the present study

I *Apparently healthy subjects* This group numbered 1706 individuals and comprised those who were reported to be healthy and not taking any drugs The criteria for exclusion have been defined elsewhere (15) Furthermore all had an ESR below 20 mm/h Subjects with a history of renal stones were not included since one of the hypotheses was that disturbances of calcium metabolism could be expected in stone disease

II *Renal stone formers* A total of 318 individuals (13 7%) had a history of renal stone disease (25) 133 of them had recurrent stones Disease was considered to be active if incidents had occurred within the last five years This was the case in 179 subjects whereas 92 of whom had only experienced a single stone whereas 41 had passed multiple (≥4) stones Among individuals with active stone disease only those 174 were included who were otherwise apparently healthy according to the criteria above

III *Controls* An apparently healthy control individual

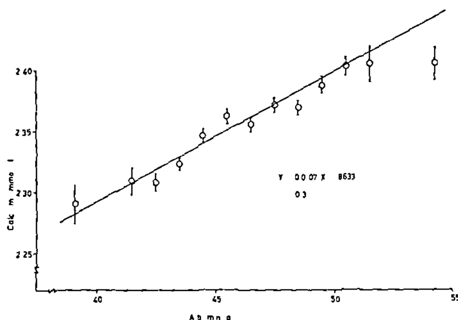


Fig 1 Relat onsh p betwe serum calcium and album n 1 706 apparently healthy m ddle aged men Symbols represent mean calc um va ues (+S E M) for each g/l n album n The lowest albumin value is the mean all values <41 g/l and the highest is the mean of value >52 g/l

from group I born in the same year and exam ned on the same day as the index person was selected for each act ve stone former

METHODS

Venous blood samples were drawn w th the subjects in the supine pos tion in the morn ng after an overnight fast

S calciu i was nalysed in a cont nuous flow system equ pped with an Eppendorf flame photo neter

S albun n was analysed with a bromcres l green b nd techn que (4) and cal brated w th punf ed album n solutions (Kab Stockholm S eden) A cont nuous flow system was used unt l Oct 1971 when it was replaced by a d screte analys ng system The latt r system showed less carry over bet veen samples as well as better intra and interda ly precis on After that the interda ly variation decreased from 3 1 5 5% to 1 1% (coeff c ent of var a tion)

S pl ospl ate was analysed with a molybden complexing method us ng Elon (p methylam nophenol sulphate) as the reduc ng method (1?) and adapted for a cont nuous flow system

Tl e control system of tl e lab rator The pat ent samples were analysed in blocks consisting of 20 (calcium and phosphate) or 30 (album n) samples 5 cal brat on standards 1 control sample 13 or ?3 pat ent samples fol lowed by another control sample In the beg nning of each run a carry-over series was run (16) and each sample was corrected in the computer system accord ng to the result of that series The mean values of the pat ent samples and control samples were calculated

D fferent control materials (human bovine and equ ne sera) were used for periods of d fferent length (? 13 months) Each day ? 3 d fferent controls with d fferent nominal values were included in the calcium analys s and 1 ? in the album n and phosphate analys s The interda ly variation was for calcium 1 1 2 1% (coeff c ent of vari tion) for album n 1 1 5.5% and for phosphate 2 5 9 9% It has not been possible to calculate the intradaily vari t on except for the later period of the invest ga on whe 0 ? 3 ?% (mean 1 3) was found for S cal um and 1 0 4 0% (mean 2 4) for S albumin

H stological criter a All parathyro d glands remo ed operat on were exam ned by the same experienced pa thologist whose criteria have been presented in deta elsewhere (19)

Meteorolog cal data concern ng hours of sunsh ne dur ing the three years of the health survey were obta ned from the Institute of Meteorology Un vers ty of Uppsala

Stat stical ı etl ods Student s t test for pa red and un pa red data was used for calculating d fferences between mean values and the χ^2 test for d fferences between fre quencies Accepted level of s gn f cance was $p<0.05$

RESULTS

Adjustment of total serui ı calciu ı

The correlation between serum calcium and al bumin was studied in the group of 1 706 apparent healthy men The linear relationsh p (Fig 1) was highly significant ($p<0.001$) The calcium values were adjusted for d fferences in album n concentra tion using the formula Ca (adjusted value) Ca (analysed value) 0 01071 (album n 46 1) where 0 01071 denotes the determination coefficient of the regression equation and 46 1 is the mean value fr albumin in the populat on The applicat on of th adjustment displayed only minor d fferences n the frequency d stribution of calcium with a sligh

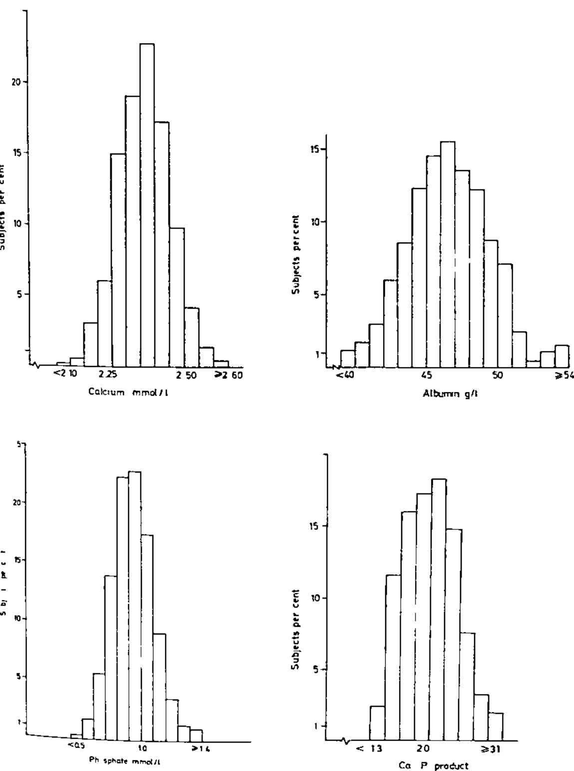


Fig 2 Frequency distributions for serum calcium albumin phosphate and Ca×P product in 2322 middle aged men

Table I *Serum calcium phosphate Ca×P product and albumin in a population of middle aged men (mean±S E M)*

	Calcium (mmol/l)	Phosphate (mmol/l)	Ca×P product (mmol/l)	Albumin (g/l)
Entire population (n=2322)	2 36±0 01	0 89±0 01	2 10±0 02	46 1±0 02
Apparently healthy (*n*=1 706)	2 36±0 01	0 89±0 01	2 10±0 02	46 1±0 02
Renal stone formers				
All (*n*=318)	2 36±0 01	0 88±0 01	2 08±0 03	46 0±0 06
Metabolically active				
Single stones (*n*=88)	2 36±0 02	0 88±0 02	2 07±0 05	46 2±0 08
Recurrent stones (*n*=86)	2 36±0 02	0 87±0 02	2 09±0 05	46 2±0 08
Multiple stones (*n*=35)	2 38±0 04	0 85±0 02	2 01±0 07	46 2±0 18

tendency towards extension on both the high and the low side Before adjustment 0 4% had a calcium value below 2 15 mmol/l compared with 0 9% after adjustment for albumin Corresponding figures for values above 2 65 mmol/l were 0 2% and 0 4% All calcium values in the following refer to adjusted values

Frequency distributions and mean values

The distributions of serum calcium phosphate Ca×P product and albumin in the entire population of 2322 middle aged men are shown in Fig 2 and mean values in Table I where data for renal stone formers from the same population are also presented The frequency distribution curves were almost identical for all groups in the table even when stone formers were compared with matched controls There was consequently no tendency towards an over representation of raised calcium values in any of the groups of stone formers Multiple stone formers had a tendency towards a lower mean value for serum phosphate This difference however was not statistically significant when compared with either the apparently healthy population or matched controls

Table II *Serum calcium and phosphate in subjects with high and low serum calcium and matched controls (mean±S E M)*

	Calcium (mmol/l)	Phosphate (mmol/l)
Subj with S-calcium <2 18 mmol/l (*n*=37)	2 14±0 01	0 86±0 03
Controls	2 38±0 02	0 87±0 03
Subj with S-calcium ≥2 55 mmol/l (*n*=28)	2 60±0 02	0 86±0 04
Controls	2 38±0 02	0 87±0 03

Correlation between serum calcium and phosphate

In the group of apparently healthy individuals there was no correlation between serum calcium and phosphate values ($r=0\ 004$) Furthermore no differences in serum phosphate appeared when only the highest and lowest calcium values were considered separately (Table II) nor was there any correlation between calcium and phosphate in any of the groups of stone formers

Seasonal variations

During the three years of the health survey there were some fluctuations in monthly mean values for all variables studied calcium phosphate Ca×P product and albumin with statistically signifi ant differences between the highest and lowest recorded mean value per month These variations over the year were as a rule inconsistent and disappeared when all three years were considered together (Fig 3) Only for serum albumin did the pattern seem to be fairly uniform for all three years The variations in albumin were not large enough to cause significant differences between the albumin-adjusted and original calcium values No correlation was found between any of the parameters and monthly hours of sunshine

Prevalence of hyperparathyroidism

Six individuals all with recurrent renal stones participating in the health survey had been previously operated on because of suspected HPT (Table III pats 1–6) All of them had then been free from stone recurrences during the observation time

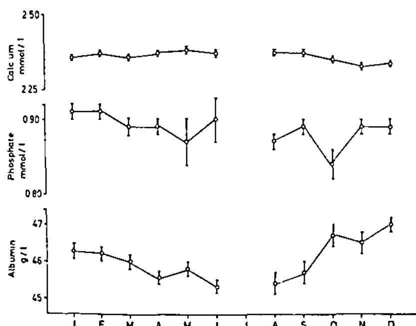


Fig 3 Monthly mean values (±S E M) for serum cal cium phosphate and albumin determined for three con secutive years in 1706 ap parently healthy middle aged men

(2–12 years) In all cases follow up disclosed con sistently normal calcium values Judging from the hospital records also one of the 446 non partici pants in the survey had been submitted to operation because of renal stones and hypercalcemia Neck exploration had revealed a parathyroid adenoma and calcium values became normal postoperatively

As can be seen from Fig 2 the serum calcium

Table III *Preoperative serum calcium values and findings at operation in ten middle aged men oper ated on for hyperparathyroidism before and after a health survey*

Pat. no	Serum calcium (mmol/l) Range	Mean	Operative findings
Before health survey			
1	3 05–3 15	3 10	Adenoma
2	2 60–2.80	2 70	Hyperplasia
3	2 65–2 75	2 65	Adenoma
4	2.50–2 70	2 65	Hyperplasia
5	2 45–2 80	2 50	Hyperplasia
6	2.35–2 45	2 40	Hyperplasia
After health survey			
7	2 65–2 75	2 70	Hyperplasia
8	2 40–2.80	2 50	Hyperplasia
9	2.55–2 65	2 60	Hyperplasia
10	2.35–2 45	2 40	Adenoma

distribution in the entire population had a very distinct margin to the right The highest recorded value was 2 73 mmol/l At the health survey eight individuals had a serum calcium of 2 65 mmol/l or more the upper normal limit in our laboratory In five of them none of whom had renal stones re peated testing showed normal values This was also the case in one man after cessation of thiazide treat ment At reexamination the remaining two still had borderline calcium values which normalized after subtotal parathyroidectomy (Table III pats 7 and 8) In another two subjects neck exploration was performed because of recurrent renal stones and hypercalciuria together with upper normal calcium values in one (no 9) and repeated gastric ulcer bleeding in the other (no 10) Stone disease was not affected in the former whereas removal of a para-thyroid adenoma in the latter caused apparent cure of both gastric and renal disorders for an observa tion time of four years

The prevalence of HPT in this population of middle aged men was 0 3% (7/2 322) if only subjects with preoperative hypercalcemia were included In the entire group of renal stone formers HPT was present in 2 2% (7/318) and among those with re-current renal stone disease in 5 3% (7/133) No case of asymptomatic hypercalcemia was found in the health survey since in all instances there had been a history of renal stone disease

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Serum and Erythrocyte Magnesium in Normal Elderly Danish People

Relationship to Blood Pressure and Serum Lipids

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ABSTRACT Serum lipids, blood pressure, and magnesium levels in serum and erythrocytes have been measured in 73 men and women aged 60 The purpose was to evaluate the relationship between the serum and erythrocyte magnesium and indicators of ischaemic heart disease The results showed significant inverse correlations between serum magnesium levels and PB, and positive significant correlation between erythrocyte magnesium and serum cholesterol

Relatively little is known about the metabolism of magnesium This lack of information may be explained by the inability to measure magnesium concentrations with high precision and accuracy The development of atomic absorption spectrophotometry in recent years has provided a simple accurate and reproduceable method to determine magnesium in biological materials (11 14 26)

Associations between hardness of drinking water and mortality from coronary heart disease are well established (2 9 20 23) These remarkable findings have been explained by the different content of calcium and magnesium in the drinking water (6 8 9 10 20 23) Bierenbaum et al (3) have shown that residents of a hard water area have significantly higher serum levels of calcium and magnesium than residents of a soft water area

Recently Seelig and Heggtveit (27) reviewed the part played by hypomagnesaemia in the development of ischaemic heart disease Furthermore it is well documented that hypercholesterolaemia and hypertriglyceridaemia are coronary risk factors (29) but there is only a little and divergent information concerning the relationship between the levels of serum magnesium and serum lipids (4 5 15)

Hypomagnesaemia has been reported in patients suffering from hypertension (1 18) This may be explained by the finding of Nemesanszky et al (21) that an increased extracellular magnesium concentration decreases vascular tone

The measurement of serum magnesium is the standard procedure for evaluating magnesium deficiency Wacker and Parisi (31) have established that cellular magnesium depletion may be observed without a concomitant lowering of the serum levels but if the erythrocyte magnesium content or the urinary excretion is normal magnesium deficiency is very unlikely

The purpose of the present investigation was to examine whether serum and erythrocyte magnesium are related to the serum lipid levels and to the blood pressure in a group of elderly Danish people

MATERIAL AND METHODS

Eighty subjects randomly selected from the population study in Glostrup of 60-year-old men and women (25) took part in the investigation Seven subjects had to be excluded since they were being treated with diuretics and/or pharmacological agents containing magnesium This left 73 subjects of whom 43 were male and 30 were female

Blood samples were drawn from all the subjects at 8-9 a m after a 12 hour fast Serum and erythrocyte magne

Table I *Measured sample values compared with corresponding reference values from the literature*

	Controls				Study sample				Coefficient of variation of duplicate measurements (%)
	Men		Women		Men		Women		
	Mean	S D	Mean	S D	Mean	S D	Mean	S D	
Serum magnesium (mg/l)	19 5[a]	1 3	19 7	1 2	19 9	1 2	19 6	1 6	0 6
Erythrocyte magnesium (mg/l)	47 5	4 5	49 4	5 2	50 9	4 7	51 0	5 2	0 5
Serum cholesterol (mmol/l)	7 26[b]	1 15	8 32[b]	1 24	6 78	0 92	7 35	2 08	1 5
Serum triglyceride (mmol/l)	1 32[b]	0 62	1 10[b]	0 41	1 51	1 19	0 97	0 39	2 6
Systolic BP	141 8	21 11	144 0	22 33	139 4	19 49	143 9	18 64	
Diastolic BP	84 5[c]	12 36	85 0[c]	12 95	88 6	11 89	89 3	11 96	
Mean BP					105 6	12 19	107 2	12 57	

[a] According to Petersen and Christiansen (22) [b] According to Dyerberg (12) According to Master et al (19)

sium were determined by atomic absorption spectrophotometry (Perkin Elmer 403) and the serum magnesium values were corrected to a constant serum protein level (7)

Serum cholesterol was measured according to Graf netter et al (13) and serum triglyceride by the method described by Laurell (16)

All the biochemical determinations were made in duplicate and the coefficients of variation are given in Table I

The BPs were measured with the subject in supine position after 15 min rest and the mean blood pressure was calculated as

$$\frac{2\times\text{diastolic BP}}{3}+\frac{1\times\text{systolic BP}}{3}$$

Test for linear correlation was used to evaluate the relationship between parameters

RESULTS

The mean values and S D of all the parameters for both sexes compared with the corresponding values in normal subjects are given in Table I

The coefficients of correlation between the parameters are given in Table II Significant inverse correlations were found between serum magnesium level and the systolic BP ($r=-0\ 31$ $p<0\ 01$) and the mean BP ($r=-0\ 27$ $p<0\ 05$) Furthermore the correlation between levels of erythrocyte mag sium and serum cholesterol ($r=0\ 25$ $p<0\ 05$) significant The remaining coefficients of corr tion were not significant

A breakdown of the 73 subjects into three gro with different mean BP showed that the mean ser creatinine values were virtually at the same le (Table III)

DISCUSSION

The subjects examined in this study were char terized as a representative sample of 60-year- Danish people (Table I)

Experts within the field of magnesium meta lism agree that the limits of the normal range serum magnesium are narrow (14 31) The me and S D reported here for serum magnesium normal subjects are in accordance with data fr other groups (17 30) The precision estimated fr duplicate measurements was 0 6% The meth used for determination of serum magnesium th seems reliable

Some studies have demonstrated that ser magnesium is lower in patients with essential hyp tension than in normal subjects (1 18) The sign

Table II *Coefficients of correlation between the parameters measured*

	Serum cholesterol	Serum triglyceride	Systolic BP	Diastolic BP	Mean BP
Serum magnesium	0 02 N S	0 04 N S	−0 31 $p<0\ 01$	−0 18 N S	−0 27 $p<0\ 05$
Erythrocyte magnesium	0 25 $p<0\ 05$	0 21 N S	−0 19 N S	−0 18 N S	−0 21 N S

N S = not significant ($p>0\ 05$)

Table III *Relation between blood pressure and serum creatinine level*

Diastolic BP	No of subj	Serum creatinine (mg/l) Mean	S D
Higher than x+1 S D	20	8 6	1 3
Between x+1 S D and x	26	9 5	1 7
Lower than x	27	10 4	2 2

cant inverse correlation between serum magnesium and mean BP reported here was not caused by a simultaneous change in the renal function since the breakdown of the subjects (Table III) demonstrates that the mean value of serum creatinine did not differ significantly between the three groups

The results demonstrate that the inverse correlation between BP and serum magnesium found in hypertensives (1 18) can also be demonstrated in normal elderly people The explanation for this may be that a comparatively high serum magnesium level protects against a rise in BP by lowering peripheral resistance An alternative explanation is that a relatively high BP lowers the tubular reabsorption of magnesium and consequently the serum magnesium level through its inherent tendency to expand the plasma volume

The positive significant correlation between the levels of erythrocyte magnesium and serum cholesterol found in this study is in accordance with the finding that erythrocyte magnesium is highly significantly increased in patients with heart diseases (22)

Our results suggest that alteration in magnesium metabolism may play a role in the development of ischaemic heart disease as stated by other investigators (6 24 27) besides influencing BP regulation (28) but further trials are clearly necessary

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Hypercalcemia and Parathyroid Function after Renal Transplantation

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ABSTRACT Hypercalcemia after renal transplantation (post T hypercalcemia) has been detected in 29 (16 7%) of 174 long term survivors The mean time of onset of hypercalcemia was 69 days after renal transplantation (range 3–210) In 18 patients the hypercalcemia was mild and resolved spontaneously (*transient*) from 2–65 months (mean 19) after onset In 4 patients serum calcium normalized concurrently w th rejection episodes In 7 patients the hypercal cem a was more pronounced (*permanent*) being ter minated by subtotal parathyroidectomy in 5 and persisting in 2 recipients The hypercalcemia was asymptomatic except in one patient who developed calculi in the graft and a fall in graft function all of which disappeared after parathyroidectomy At operat on the parathyroid glands showed hyper plasia except in one case with an adenoma in one of the hyperplastic glands Serum phosphorus was markedly decreased to the same extent in transiently and permanently hypercalcemic recipients Serum parathyroid hormone (S PTH) was increased in all of 5 patients with permanent and in 3 of 8 with transient post T *hypercalcemia In normocalcemic and in* transiently hypercalcemic recipients the mean S-PTH was identical but significantly lower than in the per manently hypercalcemic recipients S PTH was sup pressed to the same extent during an i v calcium in fusion in patients with post T hypercalcemia and with primary hyperparathyroidism

The occurrence of hypercalcem a after successful renal transplantation (RT) was first reported in 1964 (?0) and has s nce been described by several au thors (2 6 10 14 17 19 ?4) The reported inci dence of hypercalcemia in kidney recip ents has varied considerably and different opin ons as to the cl n cal s gnif cance and the need for subtotal para thyro dectomy have been presented We have therefore performed an analysis of the incidence and clinical course of hypercalcemia among the pa tients who received renal allografts in this hospital

PATIENTS AND METHODS

During the years 1964–74 291 pat ents (aged 9–65 years received k dney allografts and 174 were al ve w th a creati n ne concentration n serum at or below ? mg/100 ml 6 months after RT The incidence of post transplantat on (post T) hypercalcem a among the 174 long term surv vors is reported here

Immunosuppressive therapy was carried out w th aza th oprine and prednisone w th m nor changes n dosage schedule over the years Two months after RT the daily dose of prednisone was normally about 30 mg Antacid therapy with alum num hydroxide gel n moderate doses was given during the period when predn sone exceeded 40 mg/day i e for about 4–5 weeks

Serum concentrat ons of calcium phosphorus and alka line phosphatase were measured at weekly intervals for the f rst 4–8 weeks after RT and later at ntervals of ? 3 months Normal range for serum cal um (S Ca) s 9 0–10 8 mg/100 ml for serum phosphorus 2 7–4 7 mg/100 ml and for alkal ne phosphatase 80–??0 U/l Hypercalcem a is here def ned as serum calc um concentration above 10 8 mg/100 ml and hypophosphatem a as serum concentration of phosphorus below 2 7 mg/100 ml in two consecutive blood samples

The serum concentration of parathyro d hormone (S PTH) was measured by a rad o mmunoassay after ex tract on of the hormone from serum by adsorpt on to and elut on f om a m crofine prec p tated s l a (Quso G 3?) prov d ng a hormone concentrat on 3 ? t mes higher in ex tract than n serum (5) The antibody used was ant bov ne PTH AS ?11/32 Bovine PTH was used for ? I label ng The coeff c ent of variat on n per cent was 6 for measu e ments above and 16 for measurements with n the normal range (30–105 pg/ml bovine equ valents MRC bPTH standard 71/3?4) The sens t v ty of the assay was 10 pg bPTH present in the ncubat on m xture

Calcium infus on with s multaneous measurement of S PTH was performed in 3 patients w h permanent

Table I *Yearly incidence of hypercalcemia after renal transplantation and mean duration of dialysis*

	Recipients		Mean duration of dialysis for all 174 recipients (mo)
	At risk	With hyper calcemia	
1964–67	21	7 (33 3%)	3 3
1968	23	3 (13 0%)	4 0
1969	23	3 (13 0%)	5 3
1970	31	4 (7 8%)	5 9
1971	25	4 (16 0%)	6 0
1972	19	6 (31 6%)	7 0
1973	21	2 (9 5%)	9 0
1974	11	0 (0 0%)	8 2
1964–74	174	29 (16 7%)	5 8

post T hypercalcemia and in 3 patients with primary hyperparathyroidism with comparable serum values of calcium and PTH I v infusion of 12 mg calcium/kg b wt (as calcium laevulate) was given between 9 and 12 a m Blood samples were taken before each hour during and 2 and 20 hours after the infusion

Statistical evaluation For comparison of the incidence of post T hypercalcemia in different groups a χ^2 test was used For comparison of differences of group means Student s *t* test was used

RESULTS

Post T hypercalcemia developed in 29 (16 7%) of 174 long term surviving recipients The incidence of post T hypercalcemia decreased insignificantly over the 11 years (Table I) was not correlated with he age or sex of the recipients with the type of primary kidney disease or with the duration of dialysis before RT (Table I)

In 7 patients the hypercalcemia was permanent that is terminated by subtotal parathyroidectomy (5 patients) or persisting (2 patients) In 4 patients normalization of S Ca occurred concurrently with rejection episodes and increased prednisone doses In 18 patients the hypercalcemia was transient and resolved spontaneously

The *onset* of hypercalcemia could not be estab lished exactly in 4 patients In 2 patients hyper calcemia was present at the time of RT but ag gravated considerably after RT In the rest of the patients hypercalcemia was first detected 3–210 days (mean 69) after RT There was no difference in the mean time of onset between the permanently and transiently hypercalcemic recipients

The mean *duration* of the transient hypercalce mia was 19 months (range 2–65) None of the tran siently hypercalcemic recipients had symptom ascribable to hypercalcemia and no treatment wa given In 5 patients subtotal parathyroidectomy wa performed after 4–45 months of the hypercalcemi In one recipient a decreasing graft function an calculi in the graft developed simultaneously wit severe hypercalcemia Subtotal parathyroidectom induced normalization of all abnormalities In th other 4 patients subtotal parathyroidectomy wa performed because of long standing elevation o S Ca at or above 11 5 mg/100 ml At operation th parathyroid glands showed chief cell hyperplasia but in one case an adenoma was found within on of the hyperplastic glands The weight of th excised 3½ glands varied between 0 7 and 1 6 g

On X rays none of the hypercalcemic recipient displayed bone lesions suggesting classical osteitis fibrosa Aseptic necrosis or spontaneous fractures were found in 4 of 18 transiently and in 2 of 7 per manently hypercalcemic recipients The incidence of these bone lesions was not higher than in the normocalcemic long term survivors (22)

Biochemical findings The mean maximal S-Ca was 12 6 mg/100 ml in the permanently and 11 5 mg/100 ml in the transiently hypercalcemic patients ($p<0\ 001$) Hypophosphatemia was present in 24 of 29 patients with post T hypercalcemia when the maximal S Ca value was measured The transiently and permanently hypercalcemic recipients had identical decreased mean concentrations of serum phosphorus which were very low compared with the mean serum phosphorus of both normocalcemic recipients ($p<0\ 001$) and normal controls ($p<0\ 001$) (Table II) Elevated serum values of alkaline phos-phatase were found in 4 of 7 permanently and in 2 of 18 transiently hypercalcemic recipients ($p<0\ 01$) The mean creatinine clearance was 72 ml/min and did not differ in the two groups of hypercalcemic recipients

S PTH was above the upper normal limit in only 3 of 8 patients with transient but in all 5 with per manent hypercalcemia in whom it was measured The mean S PTH was higher in the permanently hypercalcemic recipients than in both the transient ly hypercalcemic ($p<0\ 01$) and the normocalcemic recipients with a comparable graft function ($p<0\ 01$) (Table II) In the latter two groups the mean S PTH was identical and only slightly higher than in normal subjects ($p<0\ 05$) In several transiently hypercalcemic recipients serum calcium normalized without any change in S PTH but in one case S-Ca

Table II *Serum values of calcium phosphorus parathyroid hormone (PTH) and creatinine in long term survivors after renal transplantation and in normal subjects*

	S-calcium (mg/100 ml)		S phosphorus (mg/100 ml)		S PTH (pg/ml)		S-creatinine (mg/100 ml)	
	Mean	S D	Mean	S D	Mean	S D	Mean	S D
Permanent post T hypercalcemia ($n=5$)	11 80	0 67	1 92	0 43	183	57	1 04	0 12
Transient post T hypercalcemia ($n=8$)	11 08	0 20	2 17	0 55	104	42	1 17	0 26
Normocalcemic recipients ($n=78$)	9 90	0 40	2 93	0 56	109	49	1 24	0 32
Normal subjects ($n=64$)	9 84	0 37	3 57	0 56	67	18	0 89	0 16

and S PTH normalized simultaneously S PTH decreased to normal values after subtotal parathyroidectomy

Fig 1 shows the suppression of S PTH during calcium infusion in 3 patients with primary hyperparathyroidism due to adenoma (left) and in 3 permanently *hypercalcemic recipients with comparable* preinfusion values of S-Ca and S PTH (right) Two of these patients had hyperplastic parathyroid glands but in one an adenoma was found in one of the hyperplastic glands The suppression of PTH was of the same magnitude in the two groups

DISCUSSION

Hypercalcemia will develop in some patients after renal transplantation Early reports gave an incidence of post T hypercalcemia of 50–80% (2 17 19) More recently the incidence has varied between 10 and 30% (6 10 14 24) Hampers et al

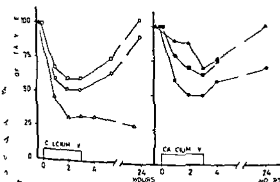


Fig 1 Suppression of parathyroid hormone (PTH) in serum by calcium infusion in three patients with primary hyperparathyroidism due to adenoma (*left*) and in three patients with post transplantation hypercalcemia (*right*) due to hyperplasia in two cases (▲—▲ ●—●) due to hyperplasia with adenoma in one (■—■)

(14) detected post T hypercalcemia in 22 (14 7%) of 150 long term survivors In the present study post T hypercalcemia was found in 29 (16 7%) of 174 long term survivors The incidence of post T hypercalcemia decreased insignificantly over the 11 years and was not correlated to the type of primary kidney disease to the age or sex of the recipients or to the duration of dialysis before transplantation

The duration and the clinical significance of post T hypercalcemia have been discussed David et al (6) and Hampers et al (14) report that parathyroidectomy is rarely necessary Geis et al (10) have performed subtotal parathyroidectomy more frequently because of elevated serum concentrations of ionized calcium in 20% of their long term survivors and at operation the parathyroid glands have shown hyperplasia However the long term effect of subtotal parathyroidectomy in these patients remains unknown In the present study the hypercalcemia was mostly modest did not cause symptoms or obvious deleterious effects in the patients and serum calcium normalized without medical or surgical interference in 18 of 29 recipients It is remarkable however that the mean duration of the mild spontaneously resolving hypercalcemia was 19 months that the duration was more than 2 years in 7 patients and the longest transient hypercalcemia lasted for 65 months In 4 patients the hypercalcemia resolved concurrently with a rejection episode treated with increased prednisone doses This is in accordance with the observation that post T hypercalcemia sometimes develops concurrently with rapid tapering of steroid doses (10 24) The high doses of steroids normally administered during the first 1–2 months after transplantation may be responsible for the late onset of post T hypercalcemia (2)

Subtotal parathyroidectomy was performed in 5 recipients 4–45 months after the onset of hypercalcemia. In only one case was the indication incontestable: severe hypercalcemia, decreasing graft function and calculi in the graft, all of which normalized after subtotal parathyroidectomy. In 4 cases the indication was persistent, moderate, unchanging elevation of S Ca and S PTH. At operation *the parathyroid glands showed hyperplasia* although an adenoma was found in one of the hyperplastic glands in one case. This is in accordance with other investigations. In fact only in 2 reported patients with post T hypercalcemia have parathyroid adenomas been found (9, 13).

David et al (6) and Kleerekoper et al (18) have reported elevated serum concentrations of PTH both in normocalcemic and in hypercalcemic long term survivors. We found elevated S PTH in all of 5 patients with permanent and in 3 of 8 with transient post T hypercalcemia. S PTH normalized after subtotal parathyroidectomy. Compared with the mean S PTH in control subjects with normal kidney function, the mean S PTH was slightly elevated both in the transiently hypercalcemic and in the normocalcemic recipients, and no difference in mean S PTH was found between the latter two groups. The mean S PTH of the transiently hypercalcemic recipients was less than the mean S PTH found in a group of primary hyperparathyroid patients with a comparable degree of hypercalcemia (unpublished data). However, S PTH was not suppressed in the transiently hypercalcemic recipients as found in patients with non parathyroid hypercalcemia (5, 15, 21). These observations lead us to assume that increased parathyroid function may not be the only etiological factor in post T hypercalcemia. The pronounced hypophosphatemia found in the hypercalcemic recipients may be a contributory factor (2, 6, 24). Phosphate supplements will often normalize post T hypercalcemia (2, 8, 24).

Hypercalcemia caused by parathyroid adenoma has been reported in patients with long standing secondary hyperparathyroidism complicating either intestinal malabsorption syndromes or chronic renal failure (3, 7, 8, 11, 12). In 1963 St Goar (3) suggested the use of the term "tertiary hyperparathyroidism" for such cases. The use of this term has, however, been debated (1). It has been argued that a certain degree of autonomy of the parathyroid glands is often found in patients with secondary renal hyperparathyroidism, in whom the elevated S PTH values cannot be suppressed to norm levels by induced hypercalcemia (1, 23, 25) and such patients the parathyroid glands have show hyperplasia. In this study S PTH was partly su pressed by i v calcium infusion both in patien with post T hypercalcemia and in primary aden matous hyperparathyroidism. Furthermore in a most all patients operated on for post T hyperca *cemia the parathyroid glands have shown hype plasia.* In fact a concurrent elevation of S Ca an S PTH indicates an abnormal feed back of calcium on PTH secretion, and there is hardly any reason t restrict the term "tertiary hyperparathyroidism" t cases with adenomas in hyperplastic parathyro glands.

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Bioavailability of Propylthiouracil Interindividual Variation and Influence of Food Intake

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ABSTRACT The bioavailability of 6-propylthiouracil (PTU) has been examined in eight healthy volunteers, with respect to interindividual variation and influence of food intake PTU was given as a single oral dose both in a fasting state and together with a standardized breakfast Numerous venous blood samples were taken during 5 hours after PTU ingestion, and the concentration of unmetabolized PTU in serum was determined by a specific gas chromatographic technique The observations indicate that the amount of PTU absorbed is subject to a large interindividual variation and that concomitant food intake may exert a minor and non systematic influence on PTU absorption Hence, the major issue in PTU therapy is the individualization of the size and interval of dosage Testing of single-dose PTU kinetics in patients would apparently be helpful

The vast majority of drugs are administered orally and many drugs are taken three or even more times per day With this schedule the drug is liable to be ingested close to meals However there is little information as to the influence of a meal on the gastrointestinal absorption of drugs Therefore we have initiated a series of studies concerning the influence of concomitant food intake on the bioavailability of different drugs In these studies we have observed that the influence of food intake sometimes can be drastic and have consequences for therapy routines (4)

Propylthiouracil (PTU) methimazole and carbimazole are thyrostatic drugs i e they inhibit synthesis of thyroid hormones within the thyroid gland 6-PTU in addition reduces the extrathyroidal conversion of thyroxine (T_4) to the more active 3 5 3′ triiodothyronine (T_3) and enhances the formation of the metabolically inactive 3,3 5 triiodothyronine (reverse T_3 rT_3) (9) As this effect is rapid while the thyrostatic effect does not lead to major reductions of the serum levels of T_3 until the thyroid hormone stores are depleted PTU may offer a therapeutic advantage In several countries however carbimazole or methimazole rather than PTU is the predominant drug in the treatment of hyperthyroidism because these two drugs are held to be more efficient than PTU Furthermore the risk of therapy failure is apparent as PTU is rapidly eliminated and hence must be administered at short intervals (1 2 6 7) With this frequency administration probably occurs close to meals which might affect the bioavailability of the drug This possibility has been explored in the present study In addition the interindividual variation in PTU bioavailability was estimated

MATERIAL AND METHODS

Eight clinically healthy volunteers five females and three males aged 18–36 weight range 55–75 kg served as test subjects Routine blood status and conventional liver function tests were normal in all After total abstention from food and liquid for ten hours (10 p m –8 a m) a polyethene cannula was inserted into an antebrachial vein and 10 ml of blood was collected (0 value—blank) Thereafter 6-PTU—300 mg in 50 mg tablets all of the same brand and batch (Tiotil Pharmacia Uppsala Sweden)—was ingested either together with 100 ml of drinking water or immediately after a standardized breakfast The breakfast prepared by a dietician was composed of 150 ml low fat milk 100 ml orange juice 1 egg 2 pieces of crisp bread 5 g margarine 20 g orange marmalade and 20 g cheese This equaled 20 g (20%) protein 17 g (35%) fat and 50 g (45%) carbohydrates and a total energy of 1 840 kJ (440 kcal) About 100 ml non sweetened black coffee

Table I *Estimates of kinetic parameters of propylthiouracil in eight healthy volunteers given a single dose on an empty stomach and together with a standardized breakfast*

t_{lag}=observed absorption delay t_{max}=observed time of peak concentration C_{max}=observed peak concentr $t_{1/2}$=estimated elimination half-life AUC=estimated area under the serum concentration curve

Subj no	t_{lag} (min)	t_{max} (min)	C_{max} (μmol/l)	$t_{1/2}$ (min)	AUC	Mean concentration (μmol/l)
Fasting						
1	30	150	17 0	55	2 310	8 8
2	40	95	81 1	85	9 820	37 6
3	15	60	40 5	60	5 310	18 8
4	55	120	32 3	75	5 260	21 7
5	10	55	40 5	85	6 010	20 6
6	10	60	30 6	75	5 280	18 2
7	50	115	23 5	60	3 100	12 3
8	15	65	49 4	95	7 690	27 0
Non fasting						
1	20	95	25 3	115	3 080	11 2
2	60	190	45 2	160	7 870	32 9
3	15	110	28 8	100	5 080	17 6
4	55	85	47 0	80	6 410	26 4
5	20	60	27 6	70	3 430	12 3
6	30	120	25 9	115*	4 930	18 2
7	20	60	31 1	50	3 170	14 7
8	15	85	48 2	85	9 310	32 9
Statistical significance (F test) of difference						
between fasting and non fasting conditions			N S	N S	N S	N S
between individuals			$p<0\ 01$	$p<0\ 01$	$p<0\ 001$	$p<0\ 001$

* Half life estimate based upon three observations only

was included The dietician or the nurse collecting the blood samples surveilled eating and intake of tablets When the tablets were taken on an empty stomach the subjects abstained from food and liquid for another two hours after drug administration

Blood samples (about 10 ml) were drawn before (0 h) and at about 15 30 45 60 75 90 105 120 150 180 210 240 and 300 min after drug ingestion The exact time (adjusted to the nearest minute) of blood sampling (when the sampling tube was half filled) was recorded and used in calculations and graphs Before each blood sampling 1–2 ml blood was obtained and discarded and after each sampling 2–4 ml 0 15 M saline was injected via the cannula The blood samples were left at room temperature for more than one but less than two hours They were then centrifuged and serum was collected and frozen at −20 C until assayed for PTU content PTU concentration in serum was assessed by a specific gas chromatographic method recently described by Schuppan et al (6)

The arithmetic and logarithmic values of the measured serum concentrations were plotted against time in diagrams from which the time to peak concentrations elimination half lives and trapezoidal AUC (area under the serum concentration curve from time of full influx (t_{lag}) to 300 min) values were calculated

Statistical differences were calculated by t tests

RESULTS

As judged from the serum concentration curves rate of elimination of PTU from blood appeare be rapid the half life being 1–2 hours Howe due to absorption delays occurring sometimes in preprandial sometimes in the postprandial st adequate determinations of the elimination h lives could not always be made (Table I Fig 1)

Within most individuals the preprandial a postprandial curves differed both with respect absorption rates peak concentrations time to p concentrations and AUC values However th was no systematic variation AUC was redu postprandially in a few subjects while the oppo was found in others In still other individuals AUC values were similar but the times to re peak concentration differed (Fig 1 Table I)

The variation between individuals was large particular the difference in AUC values was p nounced irrespective of whether pre or postpra dial curves were compared (Table I Fig 1)

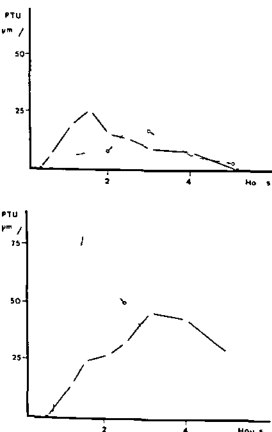


Fig 1 Serum levels of unmetabolized propylthiouracil (*PTU*) in two volunteers after a single oral dose (300 mg) given on a fasting stomach ( ) and together with a standardized breakfast (—) The amounts of PTU absorbed differed greatly (cf Table I) between subject 1 (top) and subject 2 (bottom) Food intake appeared to hasten PTU absorption in subject 1 but to delay absorption in subject 2 (cf Table I)

DISCUSSION

Adequate measurements of drug levels in blood presuppose techniques which are sufficiently specific and sensitive For PTU determinations various investigators have employed a colorimetric technique that lacks specificity not only other drugs but also several endogenous substances interfere with the assay (1 5) Moreover it is apparent that metabolites of PTU can yield reaction products which are indistinguishable from that of the parent compound (8) Indeed using the same colorimetric technique (5) two different groups have reported highly discrepant half life values (1 8) Specific methods have been devised recently (6 7) and one of these a gas-chromatographic technique by Schuppan et al (6) was adopted for the present study

Both the specific and the non specific assays have a rather low sensitivity as indicated by the finding that serum concentrations of PTU cannot be followed for more than about 4–6 hours after administration of a single oral dose (1 5–7) It follows that if absorption is delayed the plasma half life determination has to be based on very few serum values Such delays occurred sometimes in the present study and hence some of the half life values given in Table I are only rough estimates as indicated Nevertheless the estimated mean half life of 85 min agrees very well with values reported in other studies (6 7) It must be emphasized on the other hand that since no method has allowed recording of single dose blood levels of PTU for more than 4–6 hours (vide supra) there may well be an as yet undetected slow elimination phase of PTU

As judged from the AUC values the amounts of PTU absorbed differed greatly between individuals Indeed the difference between the two extreme values shown in Fig 1 was more than 5 fold This suggests that the degree of PTU absorption is subject to a pronounced interindividual variation As it has been suggested that the degree of PTU absorption is more varied in hyperthyroid than in euthyroid subjects (3) it is not unlikely that absorption differences may account for part of the well known variation in therapeutic efficacy of PTU

Another reason for this variation in efficacy could be that food intake alters the bioavailability of PTU Unquestionably most individuals displayed PTU concentration curves which differed between the pre and the postprandial state Moreover similar results were obtained when the experiments were repeated in three of the participating subjects (data not shown in Table I) Hence it is not probable that the recorded differences were purely incidental On the other hand there was no systematic variation in some individuals food intake appeared to enhance while in others it seemed to reduce the absorption of PTU

Thus there is no unequivocal answer to the question whether PTU can be ingested with a meal or should be administered on an empty stomach However as the interindividual difference in PTU absorption seemed so much greater than the intraindividual difference related to food intake the major issue in PTU therapy is not the mode of oral

administration but the individualization of dose size and dosage interval In this context testing of single-dose PTU kinetics in the patient would apparently be helpful

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Counter Regulation of Basal Insulin Secretion during Alcohol Hypoglycemia in Hypercalcemic and Normocalcemic Man

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ABSTRACT In seven normocalcemic subjects fasted for 48 hours alcohol hypoglycemia has been accompanied by a significant decline in basal insulin secretion In another six healthy subjects, fasted for two days, infusion of calcium also induced a significant fall in basal secretion of insulin However, when six healthy volunteers, fasted for two days, were infused with calcium and given alcohol simultaneously, no significant fall in insulin was recorded, even though the hypoglycemia following this combined load was of the same magnitude as in the subjects given alcohol alone The implications of these findings are discussed

It is well known from experiments in vitro that insulin secretion is highly dependent on calcium ion in the media bathing the pancreatic islets (2 4 5 13) Also in vivo extracellular calcium seems to be of importance for insulin responsiveness Accordingly it has been reported that hypercalcemia in patients with primary hyperparathyroidism augments the glucose stimulated insulin release (8) while hypocalcemia in cows results in a decreased secretion of insulin in response to glucose infusion (9) Recent studies in healthy subjects fasted over night have indicated that infusion of calcium has no significant effect on the basal secretion of insulin (3) However it is not known whether this holds true in subjects fasted for a longer period Furthermore it has not previously been studied whether hypercalcemia may affect the decline in basal insulin secretion in response to a falling glucose level in subjects fasted for a long period

In the present investigation the influence of calcium on basal insulin secretion has been studied in healthy subjects fasted for two days Moreover the effect of alcohol on basal insulin secretion and also of alcohol and calcium in combination has been investigated in healthy volunteers fasted for 48 hours

MATERIAL

The material comprised seven women aged 22–30 years and ten men 22–30 years of age All were healthy volunteers of normal weight according to the criteria for desirable weights given by the Metropolitan Life Insurance Company in Documenta Geigy in 1960 Alcohol was not abused by any of them None had glucosuria or a family history of diabetes They were all normocalcemic with serum calcium concentrations of 4 5–5 1 mEq/1000 ml

METHODS

Analytical procedures

Blood glucose was determined enzymatically with a commercial glucose oxidase preparation (Kabi Reagents Stockholm Sweden) Immunoreactive insulin in serum was assayed by a double antibody procedure essentially as described by Soeldner and Slone (17) Serum calcium was measured by a flame photometric method

Experimental model

Group 1 (nos 1–7) After a two-day fast (48 hours) seven normocalcemic subjects received 100 ml 40% alcohol orally Blood samples for analysis of glucose and insulin were collected from an antecubital vein before and after the ingestion of alcohol at intervals shown in Table I

Group 2 (nos 8–13) In another six subjects who had been fasted for two days calcium (3 75 mg/kg/60 min Calcium Sandoz 10% Sandoz AG Basel Switzerland) was infused at a constant rate for four hours into an antecubital vein In order to obtain constant infusion rates an infusion pump (Holter 908) was used One hour after commencement of the calcium infusion 100 ml 40% alcohol was given by mouth Blood samples for analysis of glucose calcium and insulin were drawn from an ante

Table I *Glucose (G mg/100 ml) and insulin concentrations (I µU/ml) in normocalcemic subjects befo and after alcohol ingestion*

Case no	Sex	Age (y)		−5	0′	30	60	90	120	150	180	210	240
1	♀	30	G	57	Alcohol ingestion	41	37	33	35	35	28	31	33
			I	5		3	4	4	4	5	4	3	4
2	♂	23	G	68		61	60	54	47	48	51	52	54
			I	2		1	1	1	2	3	2	1	2
3	♂	22	G	67		56	50	46	38	34	34	34	35
			I	6		2	2	3	4	3	2	4	3
4	♂	22	G	45		40	35	30	30	31	36	38	40
			I	7		5	4	5	4	5	5	6	6
5	♀	29	G	50		48	52	46	45	40	41	42	45
			I	8		4	5	5	3	4	4	3	3
6	♂	30	G	50		40	37	39	36	35	32	36	36
			I	7		4	3	6	4	6	6	6	4
7	♂	22	G	55		46	37	39	36	32	38	39	44
			I	5		5	5	2	3	2	4	4	4
G													
Mean				56		47	44	41	38	36	37	39	41
S D				9		8	10	8	6	6	7	7	7
Mean fall (%)						15	21	28	31	34	33	30	26
I													
Mean				5 71		3 43	3 43	3 71	3 43	4 00	3 86	3 86	3 71
S D				1 98		1 51	1 51	1 80	0 79	1 41	1 44	1 77	1 25
Mean fall (%)						40	39	37	35	24	31	33	30

cubital vein opposite the calcium infusion site before and after the alcohol ingestion as outlined in Table II

Group 3 (nos 9 10 and 14–17) In six subjects fasted for two days calcium (3 75 mg/kg/60 min) was infused i v in the same way as in group 2 However these individuals did not receive alcohol Blood samples were drawn from the opposite antecubital vein at intervals given in Table III

Calculations

When evaluating the effect of alcohol on serum insulin the following calculations were done A baseline running through the pre alcohol insulin value was drawn Then the insulin curve for a period of 240 min after the alcohol ingestion was constructed The area circumscribed by this insulin curve and the baseline was called the insulin area and for the material investigated a mean insulin area was determined Finally the effect of alcohol on serum insulin was investigated by testing whether the mean insulin area differed significantly from 0 For this purpose Student s *t* test was used

The effect of alcohol on blood glucose was tested in a similar way Furthermore these calculations were used when investigating the effect of calcium infusions alone or calcium infusions in combination with alcohol ingestion

Student s *t* test was also used when evaluating the statistical significance of differences between mean values

RESULTS

Group 1

Pre alcohol findings Fasting blood glucose showe a range of 45–68 mg/100 ml (mean 56) and insuli of 2–8 µU/ml (mean 5 71)

Post alcohol findings A marked decline i glucose was recorded after alcohol ingestion a shown in Table I and Fig 1 A Mean gluco reached a nadir (36 mg/100 ml) 150 min after alcoh ingestion Alcohol also induced a fall in insul (Table I) A nadir 40% below the pre alcohol leve was reached already 30 min after alcohol ingestio (Fig 1 B) The effect of alcohol on blood gluco and serum insulin was highly significant ($p<0\ 00$ $p<0\ 005$ respectively)

Group 2

Pre calcium infusion findings Fasting blo glucose varied between 45 and 76 mg/100 ml (mea 61) The corresponding insulin concentration wa 4–7 µU/ml (mean 5 00) and calcium concentrat 4 7–5 0 mEq/1 000 ml (mean 4 8)

Pre alcohol findings during calcium infusion Mean calcium concentration increased as a resu

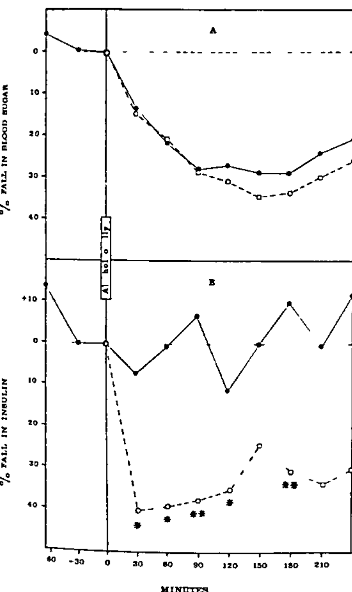


Fig 1 Mean fall in glucose (*A*) and insulin (*B*) after alcohol in seven normocalcemic subjects (O- -O) and six subjects made hypercalcemic by i v infusion of calcium between −60 and 180 min (●—●) $^*p<0.05$ $^{**}p<0.02$

of the calcium infusion to 5 6 mEq/1000 ml immediately before alcohol was given (Fig 2B) During this period no significant changes were recorded in glucose and insulin concentrations (Student s *t* test applied to paired differences)

Post-alcohol findings during calcium infusion Mean calcium concentration continued to rise during the calcium infusion to a peak of 7 1 mEq/1000 ml which was reached at the end of the calcium infusion period (Fig 2B) A decline in glucose was obtained after alcohol as shown in Table II and Fig 1A Mean glucose fell to a nadir (42 mg/100 ml) which was reached 150 min after the alcohol ingestion The decline in insulin after alcohol was slight and inconsistent as can be seen in Table II and Fig 1B When glucose and insulin areas were calculated with a view to assessing the effect of alcohol+calcium on blood glucose and serum insulin no significant effect on insulin but a highly significant effect on glucose ($p<0.001$) was recorded Moreover the mean glucose area was of the same magnitude as in group I while the mean insulin area was significantly smaller ($p<0.02$) (Fig 3)

Table II *Glucose (G mg/100 ml) insulin (I µU/ml) and calcium concentrations (C mEq/1 000 ml) healthy volunteers before during and after infusion of calcium and ingestion of alcohol*

Case no	Sex	Age (y)		−65	−60	−30	−1	0	30	60	90	120	150	180	210	240
					Infusion of calcium											
8	♂	22	G	67		61	59	Alcohol ingestion	54	54	48	50	53	42	42	48
			I	7		6	5		5	6	6	3	4	6	6	8
			C	5 0		–	5 6		–	–	6 5	–	–	7 2	–	6 8
9	♀	22	G	45		46	48		38	34	26	29	27	27	31	31
			I	4		8	8		8	6	6	7	8	6	5	9
			C	4 8		–	5 5		–	–	6 3	–	–	6 5	–	6 3
10	♀	22	G	70		66	65		67	58	55	53	54	51	56	48
			I	4		4	5		5	3	4	4	3	5	4	3
			C	–		–	–		–	–	–	–	–	–	–	–
11	♂	22	G	52		54	52		41	36	34	34	37	39	38	47
			I	4		5	4		5	6	4	4	8	6	7	9
			C	4 9		–	5 9		–	–	7 1	–	–	7 5	–	7 0
12	♀	23	G	76		76	75		69	60	57	55	54	54	63	63
			I	7		3	4		4	5	4	4	5	–	4	3
			C	4 7		–	5 3		–	–	6 1	–	–	7 1	–	6.8
13	♂	23	G	53		48	49		38	33	39	40	29	38	37	37
			I	4		3	3		1	2	5	3	1	3	2	1
			C	4 7		–	5 7		–	–	6 8	–	–	7 3	–	6.5
G																
Mean				61		59	58		51	46	43	44	42	42	45	46
S D				12		11	11		14	13	12	11	13	10	12	11
Mean fall (%)				+4		+1	0		13	22	27	26	28	28	24	21
I																
Mean				5 00		4 83	4 83		4 67	4 67	4 83	4 17	4 83	5 20	4 67	5.5[illegible]
S D				1 55		1 94	1 72		2 25	1 75	0 98	1 47	2 79	1 30	1 75	3.56
Mean fall (%)				+3		0	0		7	1	+7	12	0	+9	1	+11
C																
Mean				4 8		–	5 6		–	–	6 6	–	–	7 1	–	6 7
S D				0 1		–	0 2		–	–	0 4	–	–	0 4	–	0 3

Group 3

Pre calcium infusion findings Blood glucose ranged between 40 and 72 mg/100 ml under fasting conditions (mean 56) Insulin varied between 2 and 11 µU/ml (mean 6 67) and calcium between 4 8 and 5 1 mEq/1 000 ml (mean 4 9)

Post calcium infusion findings Serum calcium increased in almost the same way as in group 2 and reached a mean maximum of 7 2 mEq/1 000 ml at the end of the calcium infusion period (Fig 2 A) A slight fall in glucose and a concomitant decline in insulin were obtained during the infusion period (Table III and Fig 4) The effect of the calcium infusion on blood glucose and serum insulin was significant ($p<0\ 01$ $p<0\ 025$ respectively) when estimated by means of glucose and insulin areas Furthermore the mean insulin area after calcium infusion did not differ significantly from the mean insulin area after alcohol (Fig 3) The calcium infu sion alone however induced a significantly large mean insulin area than alcohol and calcium com bined ($p<0\ 05$) (Fig 3)

DISCUSSION

Previous studies have shown that after a fasting period of 36–72 hours alcohol infusion induces hypoglycemia and a rapid decline in insulin in normal as well as in obese and diabetic subjects (1 18) The present findings in seven healthy subjects given alcohol orally after a two-day fas (Fig 1) are in accordance with these results When six healthy volunteers fasted for two days were made hypercalcemic by i v calcium infusion a decline in insulin also occurred (Fig 4) Howeve when calcium infusion and alcohol administratio

Table III *Glucose insulin and calcium concentrations in healthy volunteers before and during infusion of calcium*

Abbreviations and units as in Table II

Case no	Sex	Age (y)		−5	Infusion of calcium 0	30	60	90	120	150	180	210	240
9	♀	22	G	40		38	43	34	38	41	34	33	41
			I	9		6	7	11	9	5	–	5	6
			C	4 8		–	5 4	–	–	6 4	–	–	7 2
10	♀	22	G	67		71	70	72	62	70	61	61	59
			I	9		8	6	5	5	4	5	3	4
			C	4 9		–	5.3	–	–	6 2	–	–	6 6
14	♂	26	G	67		68	63	65	61	63	61	61	63
			I	11		9	8	7	8	8	8	6	8
			C	5 0		–	5 8	–	–	7 0	–	–	8 0
15	♀	24	G	72		67	66	61	67	61	64	52	55
			I	3		2	3	3	1	1	1	2	2
			C	5 1		–	5 6	–	–	6 1	–	–	7 2
16	♀	23	G	36		40	38	38	37	33	30	28	29
			I	6		6	6	5	6	6	5	6	6
			C	5 0		–	6 0	–	–	6 6	–	–	7 2
17	♂	23	G	54		51	50	48	50	54	47	50	50
			I	2		1	2	2	1	1	1	1	2
			C	4 8		–	5 6	–	–	6.5	–	–	7 0
G													
Mean				56		56	55	53	53	54	50	48	50
SD				15		15	13	15	13	14	15	14	12
Mean fall (%)				–		0	1	5	5	4	12	16	11
I													
Mean				6 67		5 33	5 33	5 50	5 00	4 17	4 00	3 83	4 67
SD				3 61		3 20	2 34	3 21	3 41	2 79	3 00	2 14	2 42
Mean fall (%)				–		24	12	11	29	39	39	40	23
C													
Mean				4 9		–	5 6	–	–	6 5	–	–	7 2
SD				0 1		–	0 3	–	–	0 3	–	–	0.5

were performed at the same time in subjects fasted for 48 hours there was no significant decline in insulin even though the hypoglycemia following this combined load was of the same magnitude as in healthy subjects given alcohol without calcium infusion (Fig 1)

In some patients with β cell tumours no decrease in basal secretion of insulin occurs during alcohol hypoglycemia (18) In these patients the lack of a decline in insulin is probably due to an uncontrolled release of insulin by the tumour In healthy subjects made hypercalcemic by calcium infusions other explanations must be considered One is a decreased hepatic and/or peripheral extraction of insulin after alcohol ingestion in hypercalcemic compared with normocalcemic subjects Although it has been reported that the hepatic extraction of insulin may vary considerably under various conditions in dogs (7) monkeys (6) and man (6) it is not known whether the combined effect of alcohol and alterations in extracellular calcium may significantly affect the hepatic or peripheral extraction of insulin Therefore this explanation can be neither supported nor rejected

An alternative explanation for the lack of a decline in insulin after alcohol in hypercalcemic subjects could be a change in the reactivity of the β-cells to hypoglycemia Previous studies in isolated pancreatic β cells have indicated that glucose stimulation leads to an increased uptake of calcium into the β cells (11 12) Moreover it has been postulated that calcium uptake and its subsequent interaction with microtubular protein triggers emiocytosis of β cell secretory granules and the release

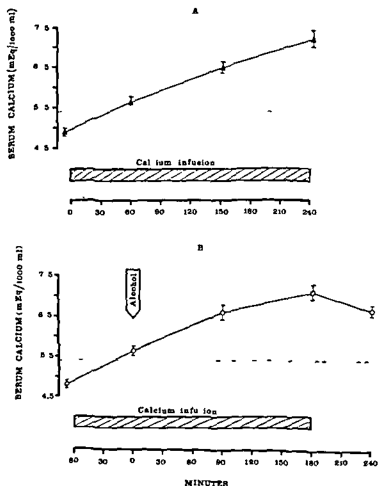


Fig 2 Calcium concentrations in health subjects in response to i v infusion c calcium *with (B) and without (A) ingestio* of alcohol (mean ±S E M)

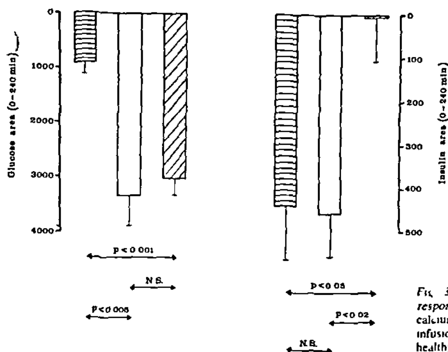


Fig 3 Glucose and insulin areas in *response to alcohol ingestion* (□) calcium infusion (▤) and calcium infusion+alcohol ingestion (▨) in healthy subjects (mean ±S E M)

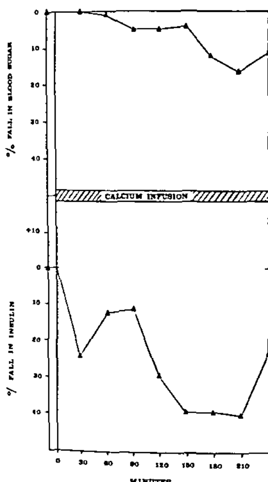


Fig 4 Mean fall in glucose and insulin after i v infusion of calcium in six healthy volunteers

of insulin (10 12) Palmer and Ensinck (14) recently reported that ethanol induced hypoglycemia was accompanied by an increase in plasma glucagon in healthy subjects fasted for 56 hours It is well documented that exogenous glucagon can stimulate insulin secretion even at very low doses (15 16) Furthermore it has been suggested that this effect of glucagon could be mediated by stimulating the uptake of calcium into the β cells (16) However in fasting normocalcemic subjects the effect of endogenous glucagon on insulin secretion might be too weak to be measurable during alcohol hypoglycemia When on the other hand alcohol is given to a fasting subject with hypercalcemia glucagon might perhaps induce a measurable increase in the secretion of insulin An increased uptake of calcium into the β cells induced by a rise in glucagon could perhaps explain the lack of a decline in insulin during alcohol hypoglycemia seen in hypercalcemic subjects in this investigation

ACKNOWLEDGEMENT

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Amylase, Hepatic Enzymes and Bilirubin in Serum of Chronic Alcoholics

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ABSTRACT The serum concentration of bilirubin and the activities of aspartate aminotransferase (ASAT, GOT) alanine aminotransferase (ALAT, GPT) γ glutamyltransferase (GT) total amylase and pancreatic isoamylase have been determined in serum of 182 male chronic alcoholics Twelve per cent had abnormally high levels of bilirubin, 73% increased activity of S-ASAT, 50% increased S ALAT, and 69% increased S GT The highest values were often found after 5–20 years of well documented alcoholism Some patients with alcoholism of more than 20 years' duration displayed a slight tendency towards normalization of the activities For all para meters the scatter around the mean was greater in the patients than in the controls Patients who had had attacks of delirium showed slightly higher S ASAT and S-ALAT than other alcoholics Deter mination of S-ALAT and S-bilirubin did not add to the cases with abnormal laboratory tests demon strated by the combination of S ASAT and S GT In 14 patients the above mentioned parameters were within normal limits, even though severe alcoholism had lasted for many years Isoamylase determination disclosed 20% to have decreased activity of pan creatic isoamylases in serum whereas only 6% had low total serum amylase activity

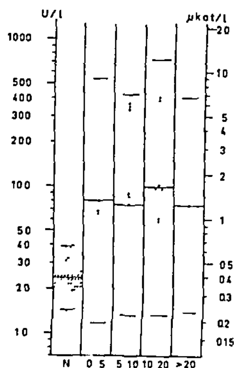
In non alcoholic individuals a single intake of 3 g ethanol/kg b wt had been found to increase the serum activity of aspartate aminotransferase (ASAT GOT) on the following day with a second peak 8 days after the intake (2) The same applies in alcohol addicts of varying severity (1 16 24) The elevation of S ASAT activity is frequently more pronounced than that of alanine aminotrans ferase (ALAT GPT) The serum activities of the aminotransferases may continue to increase for some days after ethanol intake especially in persons developing delirium tremens a phenom enon known as delayed increase (19 25) The elevation of S ALAT has been found to be more pronounced in patients who have had several attacks of delirium tremens than in those without any such attacks (25) Increased serum activities of γ glutamyltransferase (GT) have also been reported in chronic alcoholics (13) Pancreatitis chronic as well as acute with disturbed exocrine pancreatic function is often found in alcoholics (3 26 30)

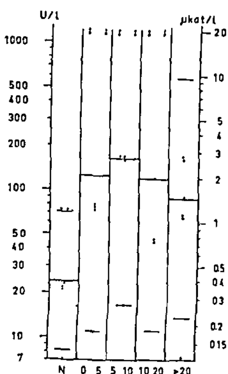
The present paper evaluates the serum con centrations of some commonly used laboratory parameters on hepatic cellular dysfunction as well as total and pancreatic serum amylase activities in an objectively defined series of persons with chronic alcoholism of varying duration

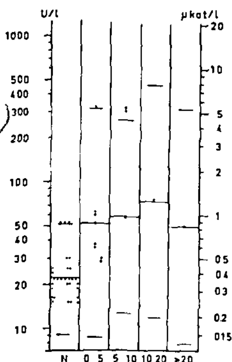
MATERIAL AND METHODS

The total patient material consisted of 182 male in patients aged 25–72 years at the Alcohol Clinic The patients had been hospitalized for detoxication social or psychiatric rehabilitation because of acute ethanol intoxication All fulfilled the criteria of γ alcoholism ac cording to the classification of Jellinek (10 11) and showed the three cardinal symptoms of alcoholism ac cording to Kaij (14) i e uncontrolled drinking black outs and restorer All patients except 3 were registered with the Temperance Board 48 had had one or more attacks of delirium tremens but not on the present ad mission None had a diagnosis of liver cirrhosis 17 had earlier had one or more attacks of acute pancreatitis Blood samples were drawn almost exclusively on the day after admission and revealed the highest recorded values of S bilirubin S ASAT S ALAT and S-GT

The reference group for normal values comprised at least 97 male shipyard workers aged 19–63 years Blood samples were collected during the same period as from the







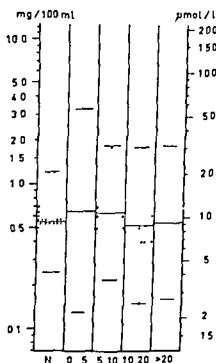
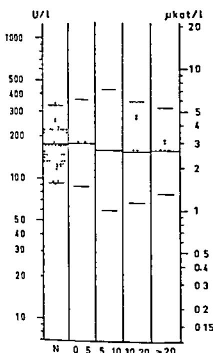
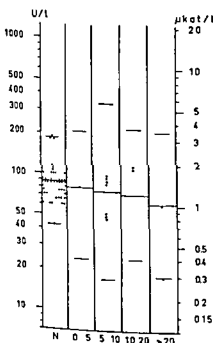


Fig 1 Serum concentration (mean ±2 S D) in normals and chronic alcoholics with disease duration of 0–5 5–10 10–20 and more than 20 years





patients The samples were analysed within 20 hours after collection

Bilirubin was determined in a Technicon AutoAnalyzer with a modification of the method by Jendrassik and Grof (12) The serum activities of ASAT and ALAT were determined with an LKB Reaction Rate Analyzer 8600 at 35°C using reagents from Kabi Stockholm Sweden the results however are given in U/l (37°) and µkat/l (37°) GT activity was determined with γ glutamyl p-nitroanilide as substrate according to Orlowski and Meister (21) and serum amylase activity with Phadebas® Amylase Test (Pharmacia Uppsala Sweden) essentially as recommended by the manufacturer Agarose gel electrophoresis was used to separate and detect the salivary and pancreatic isoamylases of serum (28) The contribution from each of these two groups of isoamylases to the serum amylase activity was determined by densitometric scanning

The patients were divided into groups according to the duration of alcoholism 0–5 5–10 10–20 and more than 20 years i e years after fulfillment of the criteria of chronic alcoholism and will be referred to below as groups 0–5 5–10 10–20 and >20

As all the parameters of the various groups showed an approximately normal distribution when transferred to logarithmic values (Fig 1) the logarithms were used in the statistical analysis The normal ranges were defined as the arithmetic mean ±2 S D of the reference group (calculated on the logarithms) Before comparing the means of the various groups with *t* analysis the variances were tested for equality with variance analysis when not equal the performance of the *t* test was modified accordingly

RESULTS

The bilirubin determinations in the reference group and the groups of chronic alcoholics gave identical arithmetic means (Fig 1) but the S D was significantly higher in all groups of alcoholics than in the reference group ($p \leq 0.05$) All groups of alcoholics had single pathologically elevated values most frequently in groups 0–5 and 10–20 The overall frequency of pathologic values among the alcoholics was 12%

In all groups of alcoholics the mean values of S ASAT and S ALAT were significantly increased p in all cases being <0 001 (Fig 1) On an individual basis 73 and 53% had increased activities of S ASAT and S ALAT respectively Here the highest activities were found in group 10–20 beyond that the means became somewhat lower though the difference was not significant for S ASAT and almost significant for S ALAT ($p \approx 0.05$) The range among the alcoholics was very wide covering the field from the lower normal limit to about 900 U/l (15 µkat/l) for both enzymes In

all groups of alcoholics the S D was significantly higher than in the normals p always being <0 001

Of the alcoholics 69% had pathologically high S GT activities the mean serum activities being significantly higher in all groups (p always <0 001) The highest mean was found in group 5–10 i e somewhat earlier than for S ASAT and S ALAT (Fig 1) Long standing alcoholism showed a tendency towards normalization of S-GT activities the mean value in group >20 being significantly lower than in group 5–10 ($p \simeq 0$ 01) The mean for group 10–20 was intermediate The range was extreme with values as high as 3 500 U/l (59 μkat/l) The S D in the four patient groups was significantly higher than in the controls (p always ≤0 01)

There were 14 alcoholics in whom all the tests were normal Everyone of them had a very well documented alcoholism 5 had been alcoholics for more than 20 years 3 had had delirium and together they had been hospitalized 74 times due to alcoholism in the past five years

Dividing the patients into two groups one with previous delirium attacks (n=48) and one without (n=126) disclosed a higher mean for S ASAT in the delirium group 156 U/l (2 60 μkat/l) than in the group without delirium 116 U/l (1 94 μkat/l) In the reference group the mean activity was 24 U/l (0 40 μkat/l) Concerning S ALAT activity the same relations were found with 111 U/l (1 85 μkat/l) in the delirium group 86 U/l (1 43 μkat/l) in the non-delirium group and 23 U/l (0 38 μkat/l) in the controls For the other parameters no such correlation was found In no case did the results of the laboratory tests correlate to the ages of the patients

A highly significant correlation was found between the serum activities of ASAT and ALAT (r=0 84 p<0 001) There was also a correlation between S ASAT and S-GT activity (r=0 60 p<0 001) A low grade but still significant correlation was found between S ASAT and S bilirubin (p<0 001 r=0 23)

The determinations of total serum amylase activity did not reveal any significant differences between means There was a possible tendency for the scatter of the individual determinations to be somewhat greater in group 5–10 compared with the control group ($p \simeq 0$ 05) The mean for pancreatic isoamylases of serum was decreased in the groups with long standing alcoholism (Fig 1) compared with the reference group p being ≈0 05 for group 10–20 and ≤0 05 for group >20 The frequency of subnormal serum activities of the pancreatic amylase was 2% in the reference group 12% in alcoholics in group 0–5 19% in group 5–10 23% in group 10–20 and 26% in group >20 The frequency of abnormally high activities of the pancreatic isoamylases in serum was not increased In all groups of alcoholics the S D was higher than in the reference group (for all groups p<0 001)

DISCUSSION

The reference group comprised a population of subjectively healthy adult males without previous control of their drinking habits This may explain their somewhat higher S GT activity compared with the values of reference individuals of both sexes normally used in our hospital (mean 14 4 U/l (0 μkat/l) upper normal limit 44 U/l (0 74 μkat/l))

Hyperbilirubinemia in chronic alcoholics has been reported earlier (31) We found no significant difference from the reference group but a few single pathologically high values in all groups These individuals always had pathological values of at least one of the enzyme tests used to indicate hepatic cellular damage

The high frequency (73%) of elevated activities of S ASAT agrees with the 73% reported by Thale (29) for a group of alcoholics as well as with results of others (16 23 31) The activity of S ASAT most probably reflects membrane leakage whereas S ALAT activity reflects cell death which could be another explanation for the findings We found patients (4 5%) whose only pathological value was an elevation of S ASAT Also Konttinen et al (16) have reported solitary elevation of S ASAT in alcoholics Several authors (16 25) have noticed that S ASAT is abnormal more often than S ALAT which could indicate that S ASAT is a more sensitive test of liver damage in alcoholics than S ALAT or that liberation of ASAT to serum also occurs in other tissues due to the damage to e g skeletal and heart muscle that is commonly found in alcoholics (4 5 6 7 8 9 15 20 22 27)

As ASAT is cleared more rapidly from plasma than ALAT the higher frequency of increased S ASAT cannot be due to differences in the rate of elimination As the blood samples in almost all cases were taken on the day after admission i e as a rule on the first day of abstinence the patients were not in steady state at the time One can therefore assume that the activities of S ASAT and

S ALAT recorded in this study were almost exclusively the highest values obtainable during the current hospitalization As the correlation between S ASAT and S ALAT is strong and S ASAT is most frequently abnormal this would be the more sensitive parameter of the two on somatic damage

An elevation of S-GT considered to be a very sensitive indication of ethanol induced liver cell dysfunction (13 16 18 19 24) was abnormal in 69% of the patients studied The correlation between S ASAT and S GT was somewhat lower than between S ASAT and S ALAT One reason for this slight discrepancy may be that muscle cell damage influences S ASAT and S ALAT activity and that these enzymes reflect cell damage whereas the S GT activity is dependent on stasis in the bile capillaries

Of the laboratory parameters used in this study the combination of S ASAT and S GT was the most sensitive in detecting hepatic or extrahepatic damage due to ethanol Determination of S ALAT and S bilirubin did not reveal any further cases with abnormal hepatic laboratory data

Using the combination of S ASAT and S-GT 8% showed normal activities even though they were very grave alcoholics The reason for this somatic resistance to ethanol is obscure but might depend on genetic or dietary factors Another puzzling finding is the tendency towards normalization of the hepatic laboratory tests seen in group >20 The reason for these two phenomena might possibly be the same the presence of an increased somatic resistance to ethanol in some individuals those without this resistance being eliminated before reaching this age group The S D for the scatter around the means of the individual observations was always statistically significantly higher for the patient groups than for the reference group This reflects great variations in the degree of the alcoholics organ damage This variation might have to do with differences in ethanol intake or in resistance to the toxic effects of ethanol Another reason for the tendency towards normalization of the laboratory values in long standing alcoholism could be a reduction of the amount of liver cells A decreased ethanol intake could also explain such a finding but this explanation was contradicted by the anamnesis and the concentration of ethanol in serum recorded on arrival at the hospital

Concerning total serum amylase activity the reference group and the patient groups showed the same mean values The scatter around the means was however somewhat greater in one patient group When the masking effect of the salivary isoamylases in serum had been eliminated by the use of specific isoamylase determinations a rather high frequency of exocrine pancreatic dysfunction was revealed in chronic alcoholics The frequency increased with the duration of the alcoholic period but was not related to earlier attacks of acute pancreatitis There were also a few cases with high pancreatic amylase activities in the serum of patients in whom pancreatic disease was not diagnosed clinically The clinical diagnosis of acute or chronic relapsing pancreatitis is difficult especially in alcoholics who often complain of epigastric pains due to other reasons such as gastritis and ulcers The increased incidence of pancreatic affection in chronic alcoholism is in accordance with earlier reports (3 26 30) Whether the low activities of the pancreatic serum isoamylases are due to a permanently decreased exocrine pancreatic function after long standing alcoholism or to a temporary reduction cannot be stated as only single blood samples were studied The successive decrease in the mean with increasing disease duration supports the idea of progressive permanent damage to the pancreas On the other hand the decreased exocrine pancreatic function reflected by the low pancreatic serum isoamylase levels developed after a comparatively short period of alcoholism which might indicate a direct effect of ethanol on the pancreatic secretion as suggested by Marin et al (17) They found that i v ethanol administration reduced the pancreatic output of enzymes and bicarbonate A longitudinal study during an abstinence period could possibly provide an answer

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Accumulation of Myoinositol in Plasma and Red Cells of Diabetic Patients

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ABSTRACT The concentration of myoinositol in plasma cerebrospinal fluid and red cells and its elimination by the kidneys have been studied in 51 diabetic patients with normal or impaired kidney function, 16 non-diabetic patients with renal failure and 37 healthy controls All diabetic patients had higher red cell concentrations of myoinositol than the controls However, only in those diabetic patients who had a glomerular filtration rate considerably below normal was the plasma concentration of myoinositol higher than in controls The findings show that the rise in plasma concentration of myoinositol most probably results from a decreased glomerular filtration rate In diabetic patients, urinary excretion of myoinositol correlated with an exponential increase in glucose excretion That myoinositol accumulates in red cells of diabetic patients may be the result of its retention within these cells caused primarily by a transient, abnormal increase in the plasma concentration of myoinositol after an average meal

Myoinositol a member of the vitamin B complex is a natural constituent of food The average daily intake has been estimated to be 1 g (3 8) It is synthetized in muscle liver brain and kidneys (4 5) In the rat the major pathway for myoinositol catabolism requires initial oxidation to D glucuronate in the renal cortex (6)

Less than 2% of ingested myoinositol is normally excreted into the urine (3) In patients with renal failure even though urinary excretion is about 5-10 times the normal plasma levels are considerably higher than average (1 10) Diabetic patients excrete more than average amounts of myoinositol into the urine (3 9) It has been suggested that the diabetic kidney does not degrade myoinositol normally (3)

To study the role of the kidney in the elimination of myoinositol the concentration of myoinositol was measured in plasma red cells cerebrospinal fluid (CSF) and urine of diabetic patients nine of them with renal failure The glomerular filtration and urinary excretion of myoinositol were calculated in these patients and in healthy control subjects

PATIENTS AND METHODS

The series comprised 51 diabetic patients of whom 9 had renal failure and 16 non-diabetic patients with renal failure Thirty seven healthy persons served as controls The age range of diabetic patients and controls was 2-80 years

Samples of venous blood were collected from all patients and controls after a 12 hour's fast These samples were immediately cooled in ice water and spun for 30 min at 4080 *g* to separate red cells from plasma The red cell mass from the first centrifugation was spun again to remove all the remaining plasma so that the final cell mass was concentrated to 98 5-99%

The concentrations of myoinositol in plasma and red cells were determined by gas liquid chromatography (9 10 11) From 11 diabetic patients with normal kidney function 9 diabetic patients with renal failure and 9 healthy control subjects the concentration of myoinositol was measured in CSF and 24 hour urine samples The 24 hour urinary glucose excretion was measured by standard methods Creatinine levels in plasma and urine were determined in a Technicon AutoAnalyzer according to the manufacturer s instruction

Glomerular filtration of myoinositol was calculated as plasma concentration of myoinositol × creatinine clearance as described elsewhere (10) Urinary excretion of myoinositol was calculated in μmol/sec using the formula

$$\frac{\text{urinary myoinositol } (\mu\text{mol}/24\text{ h})}{86\,400}$$

Statistics

To analyse the significance of the difference in mean levels the Mann Whitney U test for non parametric statis

Table I *Concentrations of myoinositol (μmol/l) in plasma red cells and cerebrospinal fluid (CSF) (mea. S E M)*
Figures within parentheses = no of subjects

	Plasma	Red cells	CSF
Controls (plasma creatinine 100 μmol/l)	23±8 (37)	20±16 (37)	130±46 (9)
Diabetics (plasma creatinine 100 μmol/l)	31±14 (51)	52±32 (51) p<0 02	114±41 (9)
Diabetics (plasma creatinine 120–480 μmol/l)	172±180 (9) p<0 01	255±270 (9) p<0 001	340±440 (9) p<0 01
Uremics (plasma creatinine 120–1 500 μmol/l)	536±349 (16) p<0 001	377±290 (16) p<0 001	384±240 (9) p<0 001

p = significance of difference in mean values between test group and controls

tics was used The median value and the range were calculated for creatinine clearance glomerular filtration of myoinositol and urinary excretion of myoinositol The correlations were tested by calculating the regression coefficient *r*

RESULTS

Mean concentrations of myoinositol in plasma red cells urine and CSF

The red cell level of myoinositol was significantly higher in diabetic patients than in the controls (Table I) Moreover both plasma and red cell concentrations of myoinositol were higher in diabetic and non-diabetic patients with renal failure than in controls the difference being highly significant (p< 0 001) The plasma level of myoinositol was higher than the red cell level in non-diabetic uremic patients

The increase in CSF concentration of myoino: tol correlated with the increase in the plasma le of myoinositol (r=0 78)

Glomerular filtration and urinary excretion of myoinositol

The glomerular filtration of myoinositol per secor was higher in diabetic patients than in healthy co trols (Table II)

An increase in glomerular filtration of myoinos tol correlated to an increase in plasma concentr tion of myoinositol in patients with normal or mo erately decreased glomerular filtration rate (Fig 1

The urinary excretion of myoinositol was ir creased in the diabetic patients independently of ir paired kidney function and correlated significant to an exponential increase in urinary glucose excre tion (Fig 2)

Table II *Glomerular filtration and urinary excretion of myoinositol compared with the plasma concentra tion of myoinositol and glomerular filtration rate (GFR) (median and range)*

	GFR (ml/sec)	Plasma myoinositol (μmol/l)	Glomerular filtration of myoinositol (nmol/sec)	Urinary excretion of myoinositol (nmol/sec)	Fraction excreted (%)
Controls (n=9)	1 5 (1 0–1 7)	27 0 (14–42)	41 0 (24 0–63 0)	1 5 (0 5–5 0)	3 6
Diabetics (n=11)	1 6 (1 0–2 4)	33 0 (14–50)	50 5 (20 0–102 0)	5 5 (3 4–11 5)	10
Diabetics (n=9)	0 8 (0 7–0 9)	240 0 (32–830)	70 0 (40 5–119 0)	18 5 (5 5–25 0)	26
Uremics (n=9)	0 1 (0 02–0 25)	435 0 (135–1 260)	27 0 (20 0–90 0)	18 0 (12 0–59 0)	67

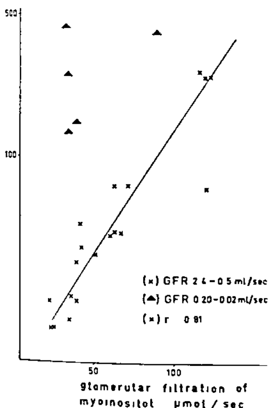


Fig 1 Correlation between glomerular filtration of myoinositol and plasma concentration of myoinositol

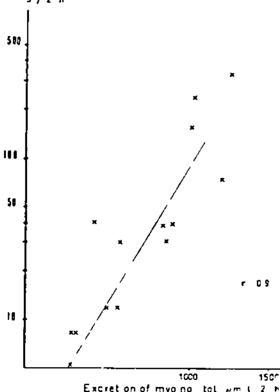


Fig 2 Correlation between urinary excretion of glucose and myoinositol in diabetic patients

DISCUSSION

An increase in plasma myoinositol concentration correlated to an increased glomerular filtration of myoinositol We observed significantly increased plasma concentrations of myoinositol only in patients with a considerably decreased glomerular filtration rate The results suggest that in any kind of renal failure even diabetic a decrease in glomerular filtration rate causes a retention of myoinositol

The red cell concentration of myoinositol in diabetic patients with normal kidney function was higher than normal The values were even increased when kidney function was impaired Clements et al (2) observed that oral administration of myoinositol caused a transient increase in plasma myoinositol in diabetic patients which was more marked than in non-diabetic controls (2) He also noted that this increase in plasma myoinositol was independent of kidney function Since food normally contains considerable amounts of myoinositol it is possible that a transient abnormal increase in the plasma level of myoinositol after an average meal results in an increased uptake and retention of myoinositol by the red blood cells in diabetic patients

The fraction of filtered myoinositol excreted in the urine was higher in the diabetic patients with normal kidney function than in the controls There was a highly significant correlation between an exponential increase in urinary glucose excretion and a linear increase in myoinositol excretion This could result from competition at the reabsorptive sites of glucose and myoinositol Myoinositol is regarded as being normally reabsorbed in the tubules and the reabsorption is blocked by saturation of the tubules with glucose and by phlorhizin (10)

The present study does not answer the question whether the diabetic kidney is incapable of degrading myoinositol normally as suggested (3 6 7) The observed accumulation of myoinositol in plasma seemed to be caused mainly by a decreased glomerular filtration rate In cases of impaired renal

function regardless of etiology an increased fraction of filtered myoinositol is excreted into the urine This presumably is due to an overall derangement of renal function including tubular dysfunction (10) In the diabetics with impaired renal function the calculated filtered amount of myoinositol was large and the tubules were thus loaded with more myoinositol than normally which could be an additional explanation for the increased excretion

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Tubular Reabsorption and Urinary Excretion of Acetoacetate and 3-Hydroxybutyrate in Normal Subjects and Juvenile Diabetics

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ABSTRACT The renal handling of acetoacetate (AA) and 3 hydroxybutyrate (3 HB) has been examined in 8 normal subjects and 7 insulin treated juvenile diabetics before and after i.v. infusion of sodium DL 3 hydroxybutyrate. In both normals and diabetics the ketone bodies were reabsorbed. At low filtration rates of AA and 3-HB the reabsorption was nearly complete. With increasing filtration rate both the tubular reabsorption rate and the urinary excretion rate of AA and 3 HB increased linearly. A maximal tubular reabsorption rate could not be demonstrated. In spite of higher filtration rates of AA and 3-HB in the diabetics, the mean reabsorption percentage of either ketone body did not differ from that found in the normals.

Studies of the renal handling of ketone bodies in man are few (2, 5, 12) and performed in circumstances where metabolism of ketone bodies and interconversion of acetoacetate (AA) and 3 hydroxybutyrate (3 HB) in the kidneys complicate ketone body clearance studies (4). In this investigation therefore renal clearance studies of AA and 3 HB were performed in normal subjects, in whom metabolism of ketone bodies in the kidneys probably is minimal. Similar studies were performed in insulin treated juvenile diabetics without reduced kidney function, as our knowledge of the renal handling of ketone bodies in these patients is scanty even though determinations of the excretion of ketone bodies in urine are used extensively in the control of the diabetic state.

MATERIAL AND METHODS

Studies were performed in 8 normal persons and 7 insulin treated juvenile diabetics. Their clinical data are given in Table I. The normals were admitted to hospital because of nervous complaints and minor diseases of the locomotor system. They had normal S-creatinine and normal urinary sediment. None of the normals had proteinuria (heat test).

In the diabetics the duration of diabetes varied between 3 and 27 years (Table I). They had normal S-creatinine and normal urinary sediment. One diabetic (no. 9) had intermittent proteinuria; in the other diabetics proteinuria was not present. At the time of the studies the diabetic state was reasonably well controlled. There were no diabetic symptoms and the patients were not acidotic. The fasting plasma glucose substance concentration is given in Table I.

The patients were studied in the fasting state in the morning before arising. Only during voiding were they allowed to stand up for a few minutes. During two or more successive periods of about 30 min clearance studies of inulin and ketone bodies were carried out simultaneously. The diabetics had been given their last insulin in the morning or afternoon of the day before the studies. One hour before the studies the patients drank 500 ml water and thereafter 200 ml at the beginning of each study period. Thus diuresis within the physiological range (1–12 ml/min) (7) was obtained. Before infusion of inulin and sodium DL 3-hydroxybutyrate a venous blood sample was taken and urine collected over a 30-min period to serve as blanks in the chemical determination of inulin in serum and urine. A priming perfusion of 35 ml of 10% inulin solution (Pure Inulin, Laevosan Gesellschaft) was then given i.v., followed by 0.5 ml/min as sustaining perfusion (8), administered by infusion pump. The concentration of inulin obtained in serum was about 30 mg/100 ml. After 30 min of constant infusion, clearance of inulin was determined in the following study periods of

Table I *Data on the diabetics and normals and their fasting plasma glucose substance concentrat* *(fP glucose)*

Pat no	Sex	Age (y)	Height (cm)	Weight (kg)	Duration of diabetes (y)	fP-glucose (mmol/l)
Normals						
1	♀	18	167	83 3		4 3
2	♀	35	165	66 0		3 9
3	♂	41	182	70 5		4 7
4	♂	26	182	75 5		5 1
5	♂	39	175	61 4		4 5
6	♂	28	181	79 0		4 3
7	♂	38	170	68 7		4 8
8	♂	25	160	49 2		4 2
Diabetics						
9	♀	43	171	64 8	27	11 5
10	♀	42	157	50 1	7	12 7
11	♀	35	160	62 4	7	17 1
12	♀	55	167	48 5	12	6 6
13	♀	19	156	55 0	9	15 3
14	♂	25	173	78 7	21	10 8
15	♂	17	178	74 9	3	7 5

Table II *Priming and sustaining perfusions of DL 3 hydroxybutyrate plasma concentration and urinar* *excretion rate of ketone bodies reabsorption of ketone bodies and inulin clearance*

Pat no	Sodium DL 3-hydroxybutyrate per fusion (1 0 mol/l)		Plasma concentration (mmol/l)		Urinary excretion rate (µmol min ⁻¹/1 73 m²)		Reabsorption (%)		Inulin clearance (ml min ⁻¹/ 1 73 m²)
	Priming (ml)	Sustaining (ml min ⁻¹)	AA	3 HB	AA	3 HB	AA	3 HB	
Normals									
1	50	2 5	0 387	1 22	26 7	23 3	34	82	104
2	50	1 5	0 173	0 441	8 46	4 58	39	87	80
		1 5	0 130	0 496	9 17	6 75	27	86	97
3	0	0	0 031	0 073	0 05	0 10	98	98	91
	50	0 5	0 139	0 269	3 79	3 20	62	83	71
4	0	0	0 091	0 015	0 08	0 11	99	93	108
	50	1 5	0 214	0 419	12 4	6 41	46	86	108
		1 0	0 162	0 287	9 11	2 67	42	90	97
5	0	0	0 036	0 015	0 09	0 09	97	93	89
	50	1 5	0 187	0 517	12 4	8 21	18	80	81
6	50	1 0	0 141	0 273	5 27	2 20	63	92	100
	50	1 5	0 223	0 419	14 4	5 98	56	90	136
7	0	0	0 032	0 017	0 02	0 06	99	96	97
	50	1 5	0 144	0 448	0 44	4 24	98	97	111
8	0	0	0 098	0 107	0 20	0 27	98	97	85
	50	1 0	0 135	0 232	1 79	0 28	83	94	80
Diabetics									
9	0	0	0 209	0 806	3 81	1 50	86	99	130
10	0	0	0 287	0 606	4 05	1 68	92	98	180
	50	1 0	0 510	1 102	28 2	36 0	64	79	154
11	0	0	0 419	1 605	25 4	66 4	72	81	217
	50	1 5	0 847	2 910	47 0	132 0	72	77	195
12	0	0	0 127	0 524	2 06	2 1	81	95	86
13	0	0	0 451	1 820	21 2	10 7	63	95	177
14	0	0	0 014	0 369	0 53	0 93	74	95	146
15	0	0	0 068	0 091	0 13	0 40	99	97	131

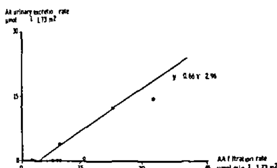


Fig 1 Correlation between urinary excretion rate and filtration rate of acetoacetate (*AA*) in normal subjects

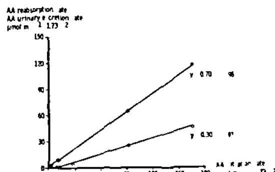


Fig 3 Tubular reabsorption rate and urinary excret on rate of acetoacetate (*AA*) in relat on to f ltrat on rate of AA in juvenile d abetics

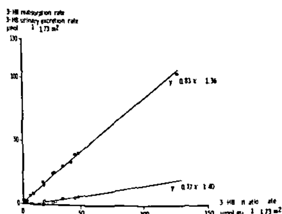


Fig 2 Tubular reabsorpt on rate and urinary excretion rate of 3 hydroxybutyrate (*3 HB*) in relation to filtrat on rate of 3 HB in normal subjects

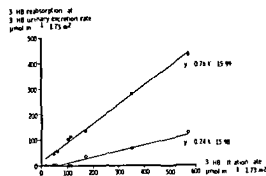


Fig 4 Tubular reabsorption rate and urinary excretion rate of 3 hydroxybutyrate (*3 HB*) in relation to filtra tion rate of 3 HB in juven le d abet cs

about 30 min The inul n determinations were carried out by the Department of Cl nical Chemistry Århus Kom munehosp tal

In order to determine clearances of AA and 3 HB at d fferent plasma levels of these substances infus on of sod um DL 3 hydroxybutyrate (1 mol/l) was given i v to some of the patients (Table II) Priming perfus on of sod um DL 3 hydroxybutyrate was 50 ml susta ning per fus on 0 5–2 5 ml/m n admin stered by infus on pump The clearance periods were started after 75 min of constant infusion During the study periods urine was col lected w thout catheter and in the m ddle of each period a venous blood sample was drawn in two tubes The first tube contain ng EDTA as ant coagulant was imme d ately placed in an icewater bath and centr fuged n an icef lled container The plasma substance concentrations of AA and 3 HB were then determ ned us ng an en zymatic micromethod (10) In the second tube the substance concentrat ons of nul n and glucose were deter m ned in the serum The substance concentrat ons of AA 3 HB inul n and glucose were also determined in the urines Urine and plasma for ketone body determinat on were buffered immed ately (10) and stored at −20 C unt l analysis the next day An O toluid ne method was used for determ nation of glucose concentration (1) and the method of Heyrovsky (3) for the measurement of inulin

In both normals and d abet cs the concentration of glucose in serum was nearly constant during the clearance periods Subtraction of the serum blank value therefore gave the serum inul n concentrat on In the d abetics the concentrat on of glucose in the ur ne specimens varied and corrections were made for interference from glucose when the concentrat on exceeded 55 mmol/l The values for plasma and urinary inulin and ketone bod es repre sent the mean value from two successive clearance periods The reabsorption rate was calculated as the d f ference between f ltrat on rate and excret on rate of AA and 3 HB The f ltrat on rate was obtained by multiplying mean plasma concentrat on of AA and 3 HB by in

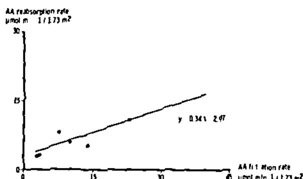


Fig 5 Correlation between tubular reabsorption rate and filtration rate of acetoacetate (*AA*) in normal subjects

clearance All values of filtration rate reabsorption rate urinary excretion rate and inulin clearance were corrected to 1 73 m² BSA

In three of the normal subjects arterial blood samples were taken at the end of the infusion of sodium DL 3 hydroxybutyrate for determination of pH pCO_2 and the substance concentration of hydrogencarbonate ion (standard) in the plasma

The Wilcoxon test for two samples Spearman s rank correlation and the method of least squares were used in the statistical analysis

RESULTS

Corresponding values of the substance concentration of ketone bodies in plasma the urinary excretion rate of ketone bodies the fraction of filtered amount of ketone bodies which is reabsorbed (the reabsorption percentage) and inulin clearance are given in Table II

The urinary excretion rates of AA and 3 HB in relation to the filtration rates of the same substances in the normals are shown in Figs 1 and 2 in the diabetics in Figs 3 and 4 It is seen that the urinary excretion rates of AA and 3 HB in normals were low at filtration rates below 10–20 µmol/min/1 73 m² virtually all of the ketone bodies filtered being reabsorbed In the diabetics too very low urinary excretion rates were found at low filtration rates With increasing filtration rate the urinary excretion rates of AA and 3 HB increased linearly The correlation between urinary excretion rate and filtration rate was significant in both normals (AA $R=0.8728$ $p<0.001$ 3 HB $R=0.9118$ $p<0.001$) and diabetics (AA $R=0.9667$ $p<0.001$ 3 HB $R=0.8833$ $p<0.01$)

The calculated reabsorption rates of AA and 3 HB in relation to the filtered loads in the normals are shown in Figs 2 and 5 The reabsorption rate rose for both ketone bodies linearly with increasing filtration rate and a significant correlation was found between reabsorption rate and filtration rate (AA $R=0.6794$ $p<0.01$ 3 HB $R=0.9963$ p 0 001) In the diabetics (Figs 3 and 4) a significant correlation was likewise found between the calculated reabsorption rate and the filtration rate for both AA and 3 HB (AA $R=0.9833$ $p<0.001$ 3 HB $R=1.0000$ $p<0.001$) the reabsorption rising linearly with increasing filtration rate The regression lines are given in Figs 1–5

The mean reabsorption percentage of AA was in the normals 66 (range 18–99) and in the diabetics 78 (range 63–99) There was no significant difference between the two mean values ($p>0.05$) The mean reabsorption percentage of 3 HB was in the normals 90 (range 80–98) and in the diabetics 91 (range 77–99) without significant difference between the mean values ($p>0.05$)

In patients 5 and 7 pH pCO_2 and P hydrogencarbonate ion in arterial blood were normal at the end of the infusion of sodium DL 3 hydroxybutyrate In patient 6 pH and P hydrogencarbonate ion were slightly elevated (7 50 and 32 mmol/l respectively) while pCO_2 was normal

DISCUSSION

The calculation of reabsorption rates of AA and 3 HB from filtration rate and urinary excretion rate is based on previous studies (12) showing that AA and 3 HB are not bound to plasma proteins and therefore fully ultrafilterable

Renal tubular reabsorption of AA and 3 HB has been found earlier in dogs on infusion of ketone bodies (6 9) and in man during starvation-exercise ketosis postabsorptive exercise ketosis and ketosis in connection with a high fat diet (2) However non specific methods used for ketone body determination impair these studies During absolute fasting recent studies in obese subjects likewise indicate a tubular mechanism of reabsorption of AA and 3 HB (5 12) without evidence of a maximal rate of tubular reabsorption (5) Starvation studies of renal arteriovenous concentration differences have shown that renal uptake of 3 HB seems to take place simultaneously with renal production of AA

(4) The results of the above mentioned studies are therefore difficult to interpret

The present studies in the normals were carried out during conditions with normal basal ketone body production where interconversion and metabolism of ketone bodies in the kidneys are probably minimal The results show that both AA and 3-HB are reabsorbed to a great extent in the kidneys the reabsorptive rates being directly related to the filtered loads A maximal tubular reabsorption rate could not be demonstrated for any of the ketone bodies However it is possible that the ketone bodies are fully reabsorbed in the tubules and the urinary excretion of ketone bodies is caused by tubular secretion In that case maximal secretory rates of AA and 3 HB were not present as the urinary excretion rates of AA and 3 HB increased with increasing filtration rate

In principle the renal handling of AA and 3 HB was identical in the juvenile diabetics and the normals In spite of higher filtration rates of AA and 3 HB in the diabetics the mean reabsorption percentage did not differ from that found in the normals Thus the tubular reabsorption of ketone bodies remained unaffected after several years of diabetes

The present studies have shown nearly complete reabsorption at low filtration rates of AA and 3 HB in both normals and diabetics From the concentration of ketone bodies in blood found in earlier studies of the diurnal variation of these substances (11 13) it can be calculated that similar low filtration rates of ketone bodies throughout the day must be present in both normals and juvenile diabetics in satisfactory diabetic regulation This explains the low 24 hour urinary excretion of AA+3 HB in normals and the nearly normal 24 hour urinary excretion of ketone bodies in juvenile diabetics during insulin treatment (13)

ACKNOWLEDGEMENT

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Immunoglobulin Levels and Autoantibodies in Epileptics on Long-term Anticonvulsant Therapy

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ABSTRACT Serum immunoglobulin (Ig) levels and various autoantibodies have been determined in 53 epileptics treated with anticonvulsant drugs for more than 10 years and in 53 controls matched for age and sex Aberrations in both IgG IgA and IgM values were demonstrated The mean IgG concentration was significantly lower in epileptics (1 217 mg/100 ml) than in controls (1 364 mg/100 ml) ($p<0\ 05$) The mean IgA value was 161 mg/100 ml in both patients and controls (*F* test, $p<0\ 01$), but abnormally low IgA values were found in 17%, and abnormally high values in 11% of the epileptics The mean IgM concentration was 157 mg/100 ml in epileptics and 117 mg/100 ml in controls (*F* test, $p<0\ 01$) Autoantibodies were found significantly more often in epileptics (26 4%) than in controls (3 8%) ($2p<0\ 002$) The occurrence of autoantibodies could not be related to alterations in the Ig levels Neither was it possible to correlate the occurrence of mitochondrial antibodies smooth muscle antibodies and antinuclear antibodies to the slightly abnormal biochemical liver parameters found in these patients Thus the abnormalities in Ig levels and the autoimmune phenomena observed in epileptics on long term anticonvulsant therapy are not intimately related

Abnormalities in both the humoral and the cellular immune function have been described in epileptic patients receiving anticonvulsant drugs Low levels of serum IgA have been reported by several authors (1 13 20 21 23) and prospective studies have shown that in some cases the low IgA values are drug induced (1 20 23) Aberrations in IgG and IgM concentrations have also been found but the results are conflicting as both elevated and decreased values have been demonstrated (1 18 21 27) Autoimmune disturbances such as th– occur-rence of autoantibodies (2) and the development of disease states resembling lupus erythematosus (17 18 19) and periarteritis nodosa (27) have also been described in patients receiving anticonvulsant therapy Similar autoimmune phenomena occur with increased frequency in patients with Ig deficiency of unknown origin (3 4 9 11) and it is possible that the autoimmune phenomena observed in epileptics are referable to Ig deficiency

With a view to examining this we investigated the serum Ig values and the occurrence of various autoantibodies in diphenylhydantoin (DPH)-treated epileptics and studied whether the presence of autoantibodies was related to serum Ig levels

Antinuclear antibodies (ANA) smooth muscle antibodies (SMA) and mitochondrial antibodies (MTA) occur in association with certain liver diseases (10 25) As abnormal liver function tests (8) and morphological changes in liver biopsies (16) have been found in DPH treated epileptics it was also investigated whether the occurrence of autoantibodies could be related to the slight biochemical changes in liver function tests which occur in patients on long term anticonvulsant therapy

MATERIAL AND METHODS

Subjects

Sera from 53 epileptic outpatients and 53 controls were investigated Sera from both groups had been stored at −20°C for a period of up to two years

The patients comprised 28 men aged 19–52 years (mean 28) and 25 women aged 18–48 years (mean 26) 24 with idiopathic and 29 with symptomatic epilepsy All patients had received anticonvulsant therapy for years They all received DPH in daily 400 mg some of them in combi

The IgG and IgA levels were lower in female than in male patients but these differences were not significant In contrast to this the mean IgM concentration was significantly higher in female (178 mg/100 ml) than in male patients (139 mg/100 ml) ($p<0.025$) No difference in the mean concentrations of different Ig classes was found in idiopathic and symptomatic epilepsy and abnormal Ig values were encountered in both groups of patients The mean IgG concentrations were lower in 12 patients receiving DPH and primidone (1 073 mg/100 ml) and in 12 patients treated with DPH in combination with carbamazepine (1 110 mg/100 ml) than in 11 patients who received only DPH (1 343 mg/100 ml) These differences were statistically significant ($p<0.01$ and $p<0.05$ respectively) No other aberrations in Ig values were related to any combination of drugs

The serum DPH concentrations ranged from 0 10 to 2 50 ng/100 ml and a positive correlation between the DPH concentrations and the serum IgG levels was found (Spearman s rank correlation test $r_s=0.33$ $p<0.025$) The DPH clearances were between 6 and 208 ml/min and a negative correlation between the DPH clearances and the IgG concentrations was disclosed (Spearman s rank correlation test $r_s=0.31$ $p<0.025$) No correlation could be established between the IgA or IgM levels and the serum DPH concentrations or DPH clearances

Autoantibodies in patients and controls

The incidence of antibodies reacting with different tissue constituents in patients and controls is shown in Table I Although the antibodies were detected with rat tissue as antigen and not with the patients own tissue they are referred to as autoantibodies The antibody titres were low but it is seen that each of the antibodies studied occurred more often in patients than in controls None of these differences were however significant SMA were of the IgG class in four patients of the IgM class in two patients and one control while one patient had SMA of both the IgG and the IgM class The ANA were of the IgM class and the MTA PA and RetA were all of the IgG class No IgA antibodies were found One or more autoantibodies were demonstrated in 14 (26 4%) of the patients and in only 2 (3 8%) of the controls (Fisher s exact test 2 $p<0.002$) Thus autoantibodies occurred more often in DPH treated epileptics than in controls Autoantibodies were found in nine (36 0%) of 25 female patients and in only five (17 7%) of 28 male patients but this female preponderance was not significant Autoantibodies occurred with almost the same frequency in idiopathic (29 1%) and symptomatic epilepsy (24 1%) The serum DPH concentration did not differ between antibody positive and antibody negative patients and the occurrence of autoantibodies was not related to any combination of anticonvulsant drugs

Relationship between autoantibodies liver function and Ig concentrations

The relationship between the biochemical liver parameters and the occurrence of MTA SMA and ANA in serum from DPH treated epileptics was studied Eleven patients had one or more of these three antibodies in serum but their liver function as assessed by biochemical liver tests did not differ from that of 42 patients without these antibodies Thus the occurrence of autoantibodies could not be associated with liver disease

It was also investigated whether the occurrence of autoantibodies was related to the Ig values in the patients Autoantibodies were demonstrated in three (33 3%) of nine patients with IgA concentrations below 37 mg/100 ml and in 11 (25%) of 44 patients with IgA concentrations above this level No autoantibodies were found in six patients with IgA concentrations above 285 mg/100 ml Autoantibodies were found in four (40%) of 10 patients with IgM values above 217 mg/100 ml and in 10 (23.3%) of 43 patients with IgM values below this level None of these differences were significant. The mean IgG concentration was 1216 mg/100 ml in antibody positive patients and 1218 mg/100 ml in antibody negative patients Thus the occurrence of autoantibodies could not be related to alterations in any of the Ig classes

Immunological aberrations i e abnormal Ig concentrations and the occurrence of autoantibodies were found in 31 patients (58%) while all parameters were normal in the remaining 22 patients (42%) Of 24 patients with idiopathic epilepsy one or more abnormal parameters were found in 18 (75%) while this was the case in only 13 (44 8%) of 29 patients with symptomatic epilepsy However this difference was not significant (χ^2 test $0.10>p<0.05$)

DISCUSSION

Our observation of serum IgA values below the normal range in 17% of DPH treated epileptics is in

agreement with previous studies in which low IgA values have been found in 20–32% of the patients (1 13 20 21 22) Others have reported only the mean IgA value in epileptics and controls and found no difference (18) It should be mentioned that the mean value of IgA did not differ in epileptics and controls in the present study either because some patients had abnormally low and others abnormally high IgA values Seager et al (20) found subnormal IgA levels prior to treatment in children who had experienced febrile convulsions earlier while those with symptomatic epilepsy had normal initial levels of serum IgA However it was also shown that the IgA levels decreased after initiation of the DPH treatment (1 20) The low serum IgG levels and the high IgM levels observed in this study are in agreement with some previous reports and in contrast to others (1 18 21 23) We found elevated IgM concentrations in DPH treated epileptics and the IgM levels were higher in female than in male patients Sorrell and Forbes (22) also found this sex difference but in their study the IgM concentrations were not elevated but depressed in treated epileptics

The pathogenesis of these drug induced Ig abnormalities is not known Salvin et al (21) found a positive correlation between IgA and IgG values and primidone levels In the present study the DPH concentration in serum correlated positively with the IgG levels but not with the IgA and IgM levels This is the reverse of what is to be expected for a drug effect However a low serum DPH concentration could be due to a high metabolic rate and thus to production of metabolic compounds in high concentration These compounds might particularly be present in patients with a high DPH clearance If these compounds exert a toxic effect on Ig synthesis this might explain why low IgG concentrations were found in patients with low DPH concentrations and high DPH clearances in our study However this would not explain why high concentrations of IgA and IgM were found in some patients

Alarcon Segovia et al (2) observed an increased incidence of ANA in patients receiving anticonvulsant drugs while Sorrell and Forbes (22) found ANA in only one of 63 DPH treated patients and Seager et al (20) could not detect ANA in any of 14 DPH treated epileptic children The present finding of an increased incidence of autoantibodies in DPH treated epileptics indicates that autoimmune disturbances occur in these patients Autoimmune phenomena also occur in patients with unexplained IgA deficiency (3) These patients lack the normal protection of IgA in the respiratory and alimentary tracts They have recurrent infections as a common symptom (14) and some of these infections might provoke the formation of certain autoantibodies (6 15) Low or undetectable levels of salivary IgA were found in DPH treated epileptics (1) Frequent infections and subsequent development of autoantibodies is thus possible in these patients However in our study the occurrence of autoantibodies was not associated with low IgA or any other Ig abnormality This suggests that the mechanisms responsible for the Ig abnormalities and the autoimmune phenomena are not intimately related MTA SMA and ANA are often found in association with liver disease (10) but we were unable to establish any relationship between the occurrence of these antibodies and the liver function in drug treated epileptics DPH has a direct effect on cell membrane permeability (26) This may cause leakage of antigens from the inside of the cells and perhaps subsequently lead to formation of antibodies against these antigens (2)

DPH treated epileptics often fail to show cutaneous delayed hypersensitivity reactions to common antigens (22 23) which may suggest that the T cell function is depressed in DPH treated epileptics A simultaneous occurrence of immunological deficiency and autoimmune phenomena has been reported (12) and it was suggested that disturbances of the thymus and other lymphoid tissues may underlie both phenomena (11) Thus it is possible that extraneous agents such as anticonvulsant drugs can cause a defect in the T cell function in some persons and then induce the development of autoantibodies and aberration in the Ig production However immunological abnormalities do not develop in all epileptics receiving long term anticonvulsant therapy and it is possible that genetic predisposition is of importance in the development of Ig deficiency and autoimmunity in drug treated epileptics

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Acute Effects of Barbiturates in Parkinson's Disease

A Preliminary Communication with Case Reports

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Parkinsonism has by tradition been treated with sedatives e g barbiturates The intention has then been to reduce the stress related component in extrapyramidal symptoms On the basis of our own clinical observations we have proposed an effect of barbiturates that seems to be specific pharmaco logically

We have noticed that pentymal sodium has visibly improved the symptoms of morbus Parkin son (MP) Our first observation of this effect was made at the Psychiatric Department when narco analysing a patient with psychoneurosis also suffer ing from MP We could reproduce the same effect in other patients with this disease without any signs of psychoneurosis On the basis of this observation we have made a minor pilot study

Pentymal sodium has been administered slowly i v in doses of 0 2–0 3 g according to body weight During the initial hypnotic phase a moderate reduc tion of the tremor seems to be correlated to the sedative effect After 1–2 hours when the patients are less sedated a reduction of tremor brady kinesia and rigidity appears The effect has usually lasted for 12–15 hours The symptoms have been estimated according to Webster s score (4) before and after the administration

MATERIAL

Case 1

A woman aged 73 with coxarthrosis since 1972 had since 2 years been subject to rigidity and prominent tremor present at rest She has managed to walk about on crutches In Aug 1975 she was treated at our department on the basis of general impairment The rigidity as well as the gait were getting worse She had no medical treatment At examination she showed a pronounced MP

Pentymal sodium 0 2 g was administered i v After two hours there was a considerable improvement According to Webster s score (4) we found a reduction of rigidi y tremor and bradykinesia from 8 to 3 points

Case 2

A woman aged 76 previously healthy In 1973 she got symptoms of MP In 1974 she was treated with L-dopa which resulted in a very moderate reduction of symptoms The disease has advanced gradually Since the spring of 1975 the patient has not been able to walk without anyone to lean upon At examination in Sept 1975 a pronounced tremor in all extremities and neck and jaws was found In addition marked bradykinesia and rigidity were ob served The patient was not able to walk She was also unable to write her name

Two hours after 0 2 g pentymal sodium i v the patient was able to walk by herself and turn around Her hand writing was readable According to Webster s score there was a reduction from 16 to 10 points

Case 3

A man aged 75 with a history of several ventricular ulcers MP started in 1970 In 1974 he had been treated with L-dopa and anticholinergic drugs which resulted in moderate improvement The patient was sent to our department in July 1975 because of vertigo We found a prominent MP According to Webster s score 21 points

Pentymal sodium 0.3 g was administered i v After 2 hours a reduction of tremor and all other symptoms was seen According to Webster s score 10 points For the first time in years the patient was able to eat properly He also managed to lift a cup to his mouth Bilateral improvement of diadochokinesia from 10 to 18 twists/10 sec

Case 4

A man aged 82 was sent to our clinic because of acute arthritis in his left knee The patient suffered from pseudo-gout At neurological examination an MP with marked rigidity was found Webster s score 2 to 3 points

Two ml pentymal sodium i v one and four hours later resulted in a reduction of symptoms from 8 to 4 points The rigidity was reduced from 2–3 to 0 points and the tremor from 1 to 0 point There was no effect on the bradykinesia

Case 5

A man aged 66 with MP since 5 years He was also suffering from chronic pyelonephritis and prostatitis Earlier treatment with anticholinergic drugs was omitted because of urinary retention He was sent to our department for L-dopa therapy At examination we found following points according to Webster s score tremor 2 points rigidity 1 point bradykinesia 1–2 points in all 12–13 points

Pentymal sodium 0 2 g was given i v One respectively four hours later there was a reduction to 7 points in all

Bilateral improvement of diadochokinesia from 10–20 twists/10 sec appeared After 30 min the patient wrote his name without any tremor

DISCUSSION

We have found that pentymal sodium has a substantial effect on idiopathic MP all modalities included The effect does not follow the sedation Some experiments with animals (2 3) speak in favour of a specific pharmacological mechanism although the pharmacological effects of barbiturates are complicated and incompletely investigated

There seems to be a possibility that the neurological systems antagonistic to the nigroneostriatal dopamine neurons are blocked by barbiturates A direct effect on the dopamine levels in the neostriatum is also possible The clinical benefit from barbiturates in this disease should be subject to further investigation

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Aldosterone Response to ACTH Stimulation in Anephric and Non-nephrectomized Patients on Regular Hemodialysis

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ABSTRACT The effect of ACTH on plasma aldosterone concentration (PAC) and plasma cortisol concentration (PCC) has been investigated in 5 anephric and 6 non nephrectomized patients on regular hemodialysis Basal PAC was significantly lower ($p<0\ 01$) in the anephric (mean 37 6 pg/ml) than in the non nephrectomized group (mean 117 5 pg/ml), whereas basal PCC (18 6 and 16 5 μg/100 ml, respectively) did not differ significantly ($p>0\ 05$) Following administration of synthetic β^{1-24} ACTH, the maximal percentage increase in PAC was significantly lower ($p<0\ 001$) in the anephric (105%) than in the non nephrectomized group (286%) The rise in PCC 118% in both groups showed no significant difference ($p>0\ 05$) The higher basal level of PAC and the more pronounced response to ACTH in non nephrectomized patients correlated with higher basal levels of plasma renin activity compared with the anephric group An influence of the remaining renin–angiotensin system on the ability to react to an ACTH stimulation is therefore suggested

In normal man the circadian aldosterone secretion is dependent on the adrenocorticotropic hormone (ACTH) and correlates well with the circadian rhythm in plasma cortisol concentration (PCC) (1 7 10) However continuous exogenous ACTH stimulation has only a brief elevating effect on the plasma aldosterone concentration (PAC) (9 18) in contrast to the persistent affect on PCC Furthermore most investigators have used very high doses of ACTH to achieve an effect on the aldosterone secretion (4 15) although Nicholls et al (16) found an increase in PAC after only 1 25 μg Finally the degree of reaction to an ACTH stimulation is dependent on the plasma potassium concentration (23) and especially on the plasma sodium concentration and the renin–angiotensin system (11 18 20 23)

In anephric patients the influence from the latter system is eliminated (3 17 21) and an excellent situation is created to study the influence of less important regulatory mechanisms on aldosterone production In the literature the results of the effect of ACTH stimulation on PAC in anephric patients are conflicting as both pronounced (12 13) and diminished increases (6 19) and no response (22) have been reported

The present study was undertaken to investigate if bilaterally nephrectomized patients were able to react to an ACTH stimulation compared with a control group and if so whether their reactivity was different from a group of non nephrectomized patients on regular hemodialysis

MATERIAL

ACTH stimulations were carried out in 11 patients with terminal renal failure on regular hemodialysis Five patients (all females) with a mean age of 42 6 years (range 37–49) were anephric The mean duration of chronic hemodialysis was 44 4 months (range 10–70) The nephrological diagnoses were chronic glomerulonephritis 2 malignant nephrosclerosis 2 polycystic kidney disease 1 Six patients (3 females and 3 males) with a mean age of 49 5 years (range 37–58) were non nephrectomized The mean duration of chronic hemodialysis was 24 0 months (range 7–52) and the nephrological diagnoses were chronic glomerulonephritis 4 polycystic kidney disease 1 chronic interstitial nephropathy 1 All patients were normotensive in good condition and did not receive hormonal treatment

Control investigations were carried out in 8 patients with chronic renal failure on regular hemodialysis Five of them (2 anephric and 3 non nephrectomized) were their

Table I. *Biochemical data of 4 anephric and 4 non nephrectomized patients during a control period*

	Aldosterone (pg/ml)			Potassium (mmol/l)			Cortisol (μg/100 ml)		
Min	Mean	S E M	%	Mean	S E M	%	Mean	S E M	%
Anephric									
0	36 75	4 60		4 33	0 21		17 67	2 38	
0	43 75	4 57	100	4 35	0 23	100	17 97	2 76	100
0	35 00	2 12		4 30	0 21		16 52	2 13	
30	33 25	1 49	86 43	4 45	0 21	103 01	12 95	1 63	74 74
60	30 75	3 66	79 93	4 57	0.24	105 79	11 12	1 83	63 94
90	34 75	4 71	90 33	4 62	0 26	106 94	9 65	1 41	55 49
120	28 50	2 32	74 08	4 60	0 27	106 48	9 72	1 30	55.89
150	30 00	3 65	77 98	4 62	0 20	106 94	9 17	1 35	52 73
180	32 00	2 16	83 18	4 60	0 19	106 48	8 80	0 96	50 60
Non nephrectomized									
0	52 50	11 28		5 00	0 23		14 13	1 03	
0	51 50	10 12	100	5 05	0 26	100	14 18	1 34	100
0	57 75	11 18		5 03	0 24		13 30	1 53	
30	60 00	9 60	111 32	5 28	0 23	105 18	12 13	1.23	87.57
60	60 00	17 78	111 32	5 38	0 27	107 17	11 20	0 99	80 81
90	70.50	16 99	130 80	5 48	0 30	109 16	9 93	1 15	71 65
120	67 25	21 33	124 77	5 48	0 30	109 16	9 80	1 26	70 71
150	60 25	15 69	111 78	5 48	0 27	109 16	8 40	0 65	60 61
180	63 25	16 36	117 35	5 50	0 26	109 56	9 72	1 78	70 13

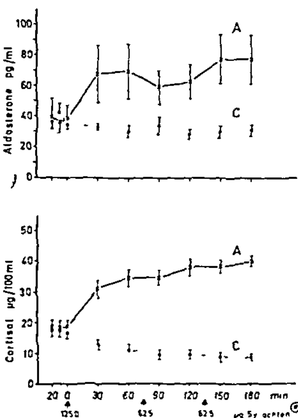


Fig. 1. Plasma aldosterone and plasma cortisol (mean ± S E M) in anephric patients during a control period (C) and following ACTH administration (A)

own controls since they were also examined after ACTH stimulation An ideal control material with all patients serving as their own controls could not be established for practical reasons chiefly due to a high transplantation frequency in our dialysis material Four of the 8 patients (all females) were anephric Their mean age was 41 3 years (range 36–58) and the mean duration of regular hemodialysis was 29 3 months (range 7–71) The diagnoses were chronic glomerulonephritis 2 polycystic kidney disease 1 malignant nephrosclerosis 1 The 4 non nephrectomized controls (2 females and 2 males) had a mean age of 48 0 years (range 37–60) and the mean duration of regular hemodialysis was 32 0 months (range 5–55) Three of them had chronic glomerulonephritis and one chronic interstitial nephropathy All were normotensive and none received any other treatment or diet than the patients in the ACTH group

METHODS

ACTH investigations were carried out at 8 a m 4 hours before the start of the hemodialysis and 3 days after the last dialysis All patients were in the supine position throughout the studies After 30 min rest 3 venous blood samples were taken at 5-min intervals before administration of ACTH At time zero 125 0 μg (12 5 IU) synthetic β^{1-24} ACTH (*Synacthen®* Ciba) was injected i v in a 500 μl solution This small volume was used to avoid expansion of the extracellular fluid volume and the injection was repeated with 62 5 μg after 75 and 135 min Blood samples were collected every 30 min during 180 min

Control experiments were carried out at the same time

Sodium (mmol/l)			Renin activity (ng/ml/h)		
Mean	S E M	%	Mean	S E M	%
136 75	1 10				
137 75	1 03	100			
137 00	0 81				
137 00	1 08	99 93			
138 00	0 57	100 66			
137 00	0 70	99 93			
136 50	0 80	99 57			
136 75	0 75	99 75			
137 75	0 63	100 48			
138 25	2 86		1 12	0 69	
138 00	2 67	100	1 25	0 67	100
138 75	2 49		0 98	0 46	
138 25	2 43	100 07	1 07	0 46	95 54
138 50	1 85	100 25	1 45	0 79	129 46
138 00	1 68	99 89	0 92	0 39	82 14
138 00	1 68	99 89	1 05	0 52	93 75
138 00	2 34	99 89	0 83	0 38	74 11
137 50	2 18	99 53	1 08	0 50	96 43

of day and in exactly the same way as the ACTH investigations except that no ACTH was given

Analyses Plasma aldosterone cortisol potassium and sodium were determined on all aliquots The plasma renin activity (PRA) was measured in all non nephrectomized and 3 of the anephric patients PAC was measured by a radioimmunoassay (24) The aldosterone antibody was a gift from the National Institute of Arthritis and Metabolic Diseases Bethesda Maryland USA The PCC was measured by competitive protein binding technique (25) Potassium and sodium by flame photometry and PRA according to the method of Harber et al (8)

RESULTS

Control investigations

In the anephric patients the mean basal PAC level was 38 5 pg/ml (S E M 3 99) with only very small variations between the individual patients (32–57 pg/ml) (Table I) During the control period the PAC was constant and nearly unchanged (Fig 1) while the mean plasma potassium concentration increased insignificantly (approximately 6%) and the mean plasma cortisol declined (approximately 50% $p<0\ 02$) The plasma sodium concentration remained constant during the control investigation

In the non nephrectomized patients the mean basal PAC was 54 0 pg/ml (S E M 10 8) with a somewhat more marked interindividual variation than in the anephric patients (Fig 2 Table I) No significant variations in the PAC were demonstrated during the control period or between individuals The plasma potassium concentration increased (approximately 10% $p>0\ 05$) and the plasma cortisol declined (approximately 30% $p<0\ 005$) The minor changes in plasma sodium concentration and PRA were insignificant (Table I)

ACTH investigations

In anephric patients the mean basal PAC before administration of ACTH was 37 6 pg/ml (S E M 9 4) and showed only a relatively small range of variation (17–71 pg/ml) as in the control group (Table II) During the ACTH injections the PAC rose to a mean maximal value of 77 1 pg/ml (S E M 16 2) (Fig 1) significantly higher ($p<0\ 01$) than basal levels and the levels in the control group The mean total increase in PAC was 105% (range 90–174) (Table II)

In the non nephrectomized patients the mean

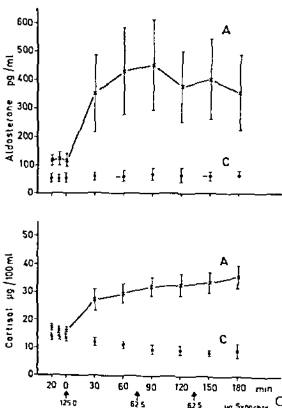


Fig 2 Plasma aldosterone and plasma cortisol (mean± S E M) in non-nephrectomized patients during a control period (C) and following ACTH administration (A)

Table II *Biochemical data of 5 anephric and 6 non nephrectomized patients before and after A[CTH] stimulation*

ACTH stimulation (µg)	Min	Aldosterone (pg/ml) Mean	S E M	%	Potassium (mmol/l) Mean	S E M	%	Cortisol (µg/100 ml) Mean	S E M	%
Anephric										
	0	39 60	12 31		5 00	0 23		18 71	2 31	
	0	35 47	6 84	100	5 00	0 22	100	18 42	2 32	100
125 0	0	37 62	9 42		5 00	0 22		18 60	2 31	
	30	67 24	18 73	179 00	5 00	0 20	100 00	31 22	2 62	16[illegible]
	60	69 87	17 20	186 00	5 32	0 35	106 40	34 73	2 86	186
62 5	90	58 69	10 92	156 24	5 51	0 39	110 20	34 81	2 54	187
	120	62 04	11 50	165 16	5 62	0 43	112 40	38 18	2 81	202
62 5	150	77 42	16 01	206 10	5 81	0 44	116 20	38 42	1 96	206
	180	77 07	16 21	205 17	5 80	0 43	116 00	40 53	1 4[illegible]	218
Non nephrectomized										
	0	116 33	44 83		4 86	0 50		17 41	1 28	
	0	121 50	48 70	100	4 88	0 54	100	16 05	1 24	100
125 0	0	115 83	47 37		4 91	0 51		15 86	1 20	
	30	350 16	136 14	297 03	5 06	0 57	103 61	27 25	3 87	165
62 5	60	428 16	152 36	363 20	5 25	0 55	107 50	29 35	3 53	178
	90	455 50	155 40	386 10	5 30	0 54	108 53	31 78	3 69	193
62 5	120	372 00	127 25	315 56	5 28	0 56	108 12	32 63	4 08	198
	150	400 50	142 90	339 73	5 36	0 49	109 76	33 43	3 49	203
	180	356 16	129 86	302 12	5 36	0 49	109 76	35 78	3 95	217

basal PAC was 117 5 pg/ml (S E M 46 7) significantly higher ($p<0$ 01) than in the anephric group and with a wider range of variation (23–309 pg/ml) During ACTH stimulation PAC rose to a mean maximal value of 455 5 pg/ml (S E M 155 4) significantly higher than in the control group ($p<0$ 01) (Fig 2) The mean total increase in PAC was 286% significantly higher than in the anephric group ($p<0$ 001) but with a much wider range of variation (181–751%) (Table II)

Fig 3 indicates that the increase in PAC after ACTH stimulation is considerably higher in non nephrectomized patients with a high basal [PAC] level than in those with a low basal PAC appr[oxi]mating that of the anephric patients

In all patients the PCC increased significa[ntly] ($p<0$ 001) by about 118% following the AC[TH] stimulation No difference was demonstrated [be]tween the two groups of patients A highly [sig]nificant correlation was demonstrated between increases in PAC and PCC in the anephric [(y=] 0 520x+0 047 $n=9$ $r=0$ 941 $p<0$ 001) as well a[s] the non nephrectomized patients (y=0 52x+10 [...] $n=9$ $r=0$ 921 $p<0$ 001)

The PRA could not be distinguished from zer[o in] the 3 anephric patients in whom it was measu[red] In the 6 non nephrectomized patients the m[ean] basal PRA was 1 96 ng/ml/h but remained const[ant] during the ACTH stimulation (Table II) In the i[ndi]vidual non nephrectomized patients a signific[ant] correlation (y=0 015x−0 228 $n=18$ $r=0$ [...] $p<0$ 001) was found between the basal PRA a[nd] basal PAC (Table III) Moreover the most p[ro]nounced PAC response to an ACTH stimulat[ion] was found in the non nephrectomized patients w[ith] the highest basal PRA levels

The rise in plasma potassium concentration w[as] insignificant ($p>0$ 05) in both groups (Table II) a[nd] the plasma sodium concentration was unch[ange]d during the investigations

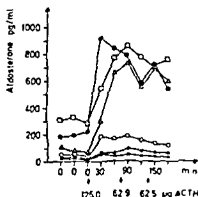


Fig 3 Plasma aldosterone concentration in 6 non nephrectomized patients following ACTH stimulation □= basal aldosterone level in anephric patients before ACTH stimulation

Sod um (mmol/l)			Renin act v ty (ng/ml/h)		
M an	S E M	%	Mean	S E M	%
139 00	0 89				
139 00	0 89	100			
139 00	0 89				
139 41	0 81	100 30			
138 6	1 24	99 73			
139 08	0 89	100 06			
138 80	1 ?4	99 86			
138 24	1 24	99 45			
138 86	1 20	99 90			
135 83	1 97		2 15	1 41	
136 00	2 06	100	1 9?	1 13	100
136 50	2 09		1 97	1 17	
137 16	2 10	100 77	1 77	1 07	87 91
137 16	2 13	100 77	1 80	1 10	89 40
136 16	1 95	99 83	2 05	1 39	101 8
136 00	06	99 80	1 70	1 16	84 43
135 50	1 83	99 33	1 85	1 25	91 88
135 83	1 9?	99 66	1 90	1 33	94 37

DISCUSSION

The l terature presents confl ct ng results concern ng the abil ty of anephr c pat ents to react with an *increase in PAC* following an ACTH st mulat on A pronounced rise in PAC has been described (1? 13) but a difference was demonstrated when the ACTH st mulat on was performed pre and post d alyt cally respectively the rise be ng more pronounced after d alys s (12) Weidmann et al (19) found a moderate to dim n shed ncrease in PAC with a good response n 3 a sl ght increase in 3 and no rise in 3 anephric patients correspond ng to the results of others (6) No changes in PAC following ACTH st mulat on were reported by Will ams et al (22) in the r 4 anephric pat ents

In non nephrectom zed pat ents w th term nal renal failure on regular hemod alysis an increase has been demonstrated in PAC in response to an ACTH st mulation (20)

In the present invest gat on the basal levels of PCC were similar in anephric and non nephrectomized patients and within normal ranges (?5) Furthermore the increase in PCC following ACTH st mulation was ident cal in the two groups of pa tients and corresponded to the levels found in other invest gations (12 19) This suggests that the adre nals in the anephric pat ents have not been damaged during nephrectomy

The PAC ncreased by approximately 100% after ACTH st mulation in our anephric patients This increase was signif cant s nce a control invest ga tion extend ng over the same per od (n wh ch ACTH stimulation was om tted) showed no s gnifi cant change in PAC and correlated well ($p<0\ 001$) with the increase in PCC Anephric pat ents can thus react with an increase n PAC follow ng ACTH stimulation conf rm ng the results of two other in *vestigat ons (12 13) compr s ng 5 and 8 pat ents* respect vely

In the non nephrectom zed pat ents w th term nal renal failure on regular hemod alys s an ncrease in PAC in response to an ACTH st mulation was demonstrated too The increase averaged ?86% (range 181 751%) and was thus more pronounced than in the anephr c pat ents although the PCC response was dent cal n the two groups (Fig 2)

Other experimental and clin cal invest gat ons have ind cated that both the sod um concentrat on and the ren n–angiotensin system (5 6 11 18 20 23) as well as the potass um concentrat on (14 19 23) may nfluence the PAC response to an ACTH st mulat on

In the present study the mean PAC response to an ACTH st mulat on n non nephrectom zed pa tients was nearly three t mes as h gh as in anephr c pat ents In the non nephrectom zed group the 3 pat ents w th low or normal basal PRA showed a PAC response as seen n the anephr c group while the rema n ng 3 non nephrectom zed pat ents with a pronounced PAC response to ACTH all had h gher basal PRA levels (F g 3)

Table III *Mean basal plasma ren n act ty (PRA) and plasma aldosterone concentrat on (PAC) levels befo e ACTH st mulat on and mean max mal n crease n PAC after ACTH st mulat on n 6 non neph ectom ed pat ents*

Range w h n parentheses

Basal PRA (µg ml/h)	PAC (pg ml) Basa	Max ncrease afte ACTH
0 4 (0 3–0 4)	3 6– 42)	64
0 6 (0 5–0 6)	3 (2 25)	43
0 8 (0 8–0 8)	52 (46– 61)	141
0 8 (0 8–0 9)	86 (78–105)	646
1 0 (0 8–1 1)	03 184–2 8)	720
5 5 (4 8–6 3)	309 (294–330)	558

In all patients the plasma potassium increased during the study (10–16%) slightly more than during the corresponding control period and significant correlations were demonstrated between the plasma potassium and the PAC and PCC respectively Therefore it cannot be established from the present data whether the effect of ACTH on aldosterone production is mediated via changes in the potassium content in the adrenal cortex as suggested by Baumber et al (2) or is a direct effect on the conversion of corticosterone to aldosterone

In conclusion anephric as well as non nephrectomized patients on regular hemodialysis are able to react with an increase in PAC following ACTH stimulation In non nephrectomized patients the increase in PAC is more pronounced than in anephric patients despite a similar increase in PCC in the two groups Thus it cannot be excluded that a remaining renin–angiotensin system somehow influences the ability of the adrenal cortex to respond to an ACTH stimulation with an increased aldosterone secretion

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Determination of Effective Orifice Area in Mitral Stenosis from Non-invasive Ultrasound Doppler Data and Mitral Flow Rate

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ABSTRACT Ten patients with mitral stenosis, but without mitral insufficiency, have been studied during cardiac catheterization The mitral orifice blood velocities the mitral pressure gradient and the mitral flow rate were determined with ultrasound manometry, and the direct Fick method, respectively The effective orifice area was calculated from the ultrasound data and the mitral flow rate The geometric orifice area was calculated from the pressure gradient and the mitral flow rate using a revised Gorlin formula A comparison of the two methods showed a correlation coefficient of 0 975 The investigation demonstrated that the ultrasound method represents an alternative to the conventional catheterization methods used for the quantification of mitral flow obstruction

The hemodynamic disturbances found in patients with mitral stenosis are a result of the increased flow obstruction in the mitral orifice In the management of patients with this disease and in the evaluation of the results of surgical treatment of the disease it is of importance to be able to quantify the magnitude of the mitral flow obstruction

If the pressure gradient–flow rate relationship in the mitral orifice is parabolic the flow obstruction is defined by a single number the effective mitral orifice area In cardiology the geometric orifice area as calculated from the Gorlin formula (1) is often used as a measure of the obstruction

Non invasive ultrasound doppler systems can theoretically determine the blood velocity in the mitral orifice (3) and it may therefore be possible to quantify the mitral flow obstruction non invasively if the mitral flow rate is known Such a method may represent an alternative to the conventional catheterization methods used to determine the parameters in the Gorlin formula

In this investigation the nature of flow in orifices of fixed geometry has been studied with ultrasound and manometry Also in 10 patients with mitral stenosis the effective mitral orifice area has been calculated from the mitral orifice blood velocity as determined with non invasive ultrasound and the mitral flow rate as determined with the direct Fick method The results have been compared with those obtained using a revised Gorlin formula (2)

The study represents another aspect of a recent investigation (3)

METHODS

Theoretical considerations

The following theoretical analysis disregards the unsteady nature of the blood flow in the mitral orifice It also disregards the fact that the mitral orifice geometry changes throughout diastole

In the steady flow of an incompressible fluid through an orifice situated between two stagnant reservoirs the viscous losses of energy become negligible compared with the losses of kinetic energy if the Reynolds number is sufficiently large (The Reynolds number for the flow in an orifice can be defined as

$$\frac{\text{(fluid velocity) (orifice diameter)}}{\text{(fluid viscosity)}} \quad)$$

In such a flow situation the kinetic energy present in the orifice jet where the fluid velocity is at its maximum i e at the vena contracta is completely dissipated in the vortices downstream of the orifice so that the pressure

gradient across the orifice is simply equal to the kinetic energy per unit mass at the vena contracta The retarding effects of the viscous boundary layer are also negligible in such a flow situation so that the fluid velocity at the vena contracta is constant over the cross section of the flow area

Thus the gradient–velocity relationship is described by eq [1]

$$\Delta P = \tfrac{1}{2}\rho V^2 \qquad [1]$$

ΔP = pressure gradient across the orifice ρ = mass density of fluid V = fluid velocity at the vena contracta subsequently called jet velocity

The discharge coefficient of an orifice is the ratio of the effective orifice area i e the flow area at the vena contracta and the geometric orifice area Thus

$$C_d = \frac{A_e}{A_g} \qquad [2]$$

C_d = discharge coefficient A_e = effective orifice area A_g = geometric orifice area

In industrial orifice flow meters C_d is relatively constant over a wide range of Reynolds numbers and its value depends mainly upon the orifice inlet geometry An orifice with a well rounded nozzle like inlet can have a C_d close to 1 0 whereas a sharp edged inlet has a C_d of about 0 6 (4) It is important to note that eq [1] can be valid for a number of different inlet geometries

For an orifice where C_d is constant and V is constant over the cross section of the vena contracta it is thus legitimate to write the following equation

$$\frac{dQ}{dt} = A_e V \qquad [3]$$

Q = orifice volume flow t = time

Combination of eq [1] and eq [3] results in the following parabolic pressure gradient–flow rate relationship

$$\Delta P = \frac{\rho}{2A_e^2}\left(\frac{dQ}{dt}\right)^2 \qquad [4]$$

ρ is a constant the pressure gradient–flow rate ...onship in the orifice is entirely defined by the magnitude of A_e Thus A_e is a measure of the flow obstruction in the orifice the smaller A_e the larger the obstruction The product C_dA_g is also a measure of the obstruction but it should be noted that A_g alone is not such a measure since C_d can be expected to vary from orifice to orifice

Eq [3] can be manipulated mathematically into a form more suitable for stenotic mitral orifices Integration of the equation over 60 sec and substitution of the mean diastolic jet velocity times the diastolic filling time for the velocity integral yields

$$A_e = \frac{Q}{V\ T} \qquad [5]$$

A_e = effective mitral orifice area (cm²) Q = mitral flow/min (cm³) V = mean diastolic jet velocity (cm/sec) T = diastolic filling time (left ventricular filling time) (sec/min) If eq [4] is valid for stenotic mitral orifices eq [5] can theoretically be used to determine A_e from non-invasive u[...] sound data if Q is known

The mitral pressure gradient obtained at catheteri[...] can also be used to determine A_e Thus combin[...] [1] and eq [5] yields

$$A_e = \frac{Q}{51\ 7\ \sqrt{\overline{\Delta P}}\ T}$$

$\overline{\sqrt{\Delta P}}$ = mean square root of diastolic mitral pre[...] gradient (mmHg$^{1/2}$) In eq [6] a value of 1/981 g[...] cm⁴ has been used for the mass density of blood

The Gorlin formula (1) in a similar form is

$$A_g = \frac{Q}{31\ T_p\ \sqrt{\overline{PWP}\ 5}}$$

A_g = geometric orifice area (cm²) $\overline{PWP}$ = mean pulmo[...] artery wedge pressure (mmHg) T_p = diastolic filling [...] *as obtained from brachial artery pressure tracing* [...] min)

On the basis of experimental work Hammerme[...] et al (2) have proposed a revised Gorlin formula [...] suitable for data obtained from simultaneous right [...] left heart catheterization

$$A_g = \frac{Q}{40\ T\ \sqrt{\overline{\Delta P}}}$$

$\overline{\Delta P}$ = mean diastolic gradient (mmHg)

The form of the time course of the pressure gra[...] in mitral stenosis is such that the difference betw[...] $\overline{\sqrt{\Delta P}}$ (eq [6]) and $\sqrt{\overline{\Delta P}}$ (eq [8]) can be expected to [...] negligible Dividing eq [6] by eq [8] yields a C_d of 0 [...] this C_d should then represent the mean discharge coe[...] cient in the patient material studied by Gorlin and Go[...] (1) Thus by dividing A_e as obtained from the ultras[...] data (eq [5]) by 0 775 the resulting A_g can be compa[...] directly with the A_g obtained from the mitral press[...] gradient (eq [8])

Ultrasound equipment

The ultrasound system was a modification of 2 1 M[...] Hewlett Packard Sound Monitor (3) and a Kay So[...] Graph 6061B Sound Spectrum Analyzer The output [...] the Sound Monitor was recorded on magnetic tape c[...] tained within the frequency analyzer

Calculation of blood velocities

The time course of the jet velocity was determined in [...] tests in the following manner The recorded ultraso[...] data were frequency analyzed and the time course of t[...] maximum frequency shift was identified The time cou[...] of the jet velocity was then calculated from the Dopp[...] equation

$$V = \frac{c\ \Delta f}{2\ f\ \cos\theta}$$

V = jet velocity (cm/sec) c = velocity of sound in tiss[...] (1 5×10⁵ cm/sec) Δf = maximum frequency shift as o[...] tained from the frequency analysis (Hz) f = frequency [...] incident ultrasonic beam (2 1 MHz) θ = angle betwe[...]

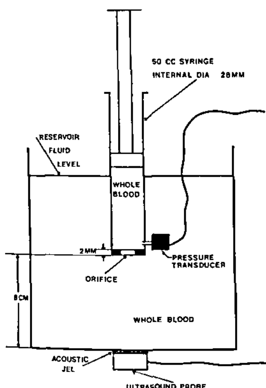


Fig 1 Schematic representation of experimental set up for in vitro testing of eq [1]

axis of incident ultrasonic beam and velocity vectors at the vena contracta $\cos\theta = 1$ assumed in all calculations

In vitro and in vivo evaluation of eq [1]

Eq [1] implies that the flow obstruction in an orifice can be quantified with a single number and that the jet velocity is constant over the cross section of the vena contracta The validity of eq [1] for the blood flow in stenotic mitral orifices is thus of crucial importance for the method under investigation In vitro and in vivo tests were therefore designed to explore the range of validity of the equation

In the in vitro tests a bolus of bank blood at room temperature was forced through a submerged orifice by manually depressing a syringe plunger (Fig 1) Orifice diameters of 8 3 5 and 1 5 mm were used All orifices had inlets rounded to a radius of 1/2 mm and an orifice thickness of 2 mm The frequency shifts due to the orifice jet were recorded via an ultrasound probe positioned so that the axis of the incident ultrasonic beam coincided with the axis of the orifice thus ensuring a value of 1 0 of the term $\cos\theta$ in the doppler equation The pressure gradient across the orifice was recorded via a pressure transducer The pressure gradient ΔP_U was calculated from eq [1] and compared with the actual pressure gradient ΔP_M as obtained from the pressure tracing

The in vivo evaluation of eq [1] was performed at Reynolds numbers considerably smaller than those expected in mitral stenosis A siliconized plastic cup with a square edged orifice with a diameter of 1 mm was implanted surgically in an anesthetized dog s abdominal aorta about 5 cm above the trifurcation (Fig 2) The wall thickness of the cup and the orifice thickness were 1/2 mm the length of the cup 8 mm and the external diameter of the cup 9 mm about the same as the internal diameter of the dog s abdominal aorta The ultrasound probe was placed in the trifurcation so that the axis of the incident ultrasonic beam coincided with the axis of the orifice acoustic contact between probe and vessel wall was maintained with an acoustic gel Simultaneous recordings were made of the frequency shifts due to the orifice jet and the pressures above and below the orifice The orifice pressure gradient ΔP_U was calculated from eq [1] and compared with the actual pressure gradient ΔP_M as obtained from the two pressure tracings

Determination of geometric and effective orifice areas in patients with mitral stenosis

The patient material consisted of 10 adult patients with mitral stenosis but without angiographic evidence of mitral insufficiency With the patients in the resting state and the right heart catheter (Cournand) in the pulmonary artery and the left heart catheter (polyethylene) in the descending aorta the cardiac output was determined during a 3 min period with the direct Fick method The catheters were then advanced to the pulmonary artery wedge position and the left ventricle respectively

In order to achieve $\cos\theta = 1$ in the doppler equation the region of the mitral orifice was scanned with the ultrasonic beam by manually moving the probe across the left anterior and lateral chest with the aid of the audio signal of the Sound Monitor the probe position that resulted in the largest frequency shifts was identified With the probe in this position the ultrasound data the pulmonary artery wedge pressure and the left ventricular pressure were recorded simultaneously An acoustic gel was used to maintain acoustic contact between probe and skin

The time course of the diastolic mitral pressure gradient was constructed by subtracting the left ventricular pressure from the pulmonary artery wedge pressure Prior to the subtraction the left ventricular pressure tracing was manually delayed 0 08 sec relative to the wedge pressure

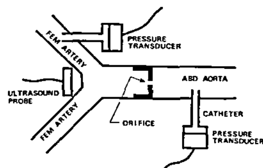


Fig 2 Schematic representation of experimental set up for in vivo testing of eq [1]

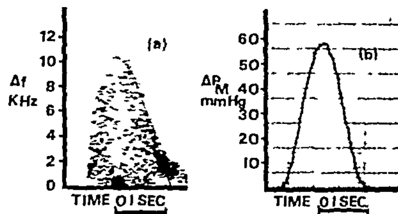


Fig 3 Concomitant data from depression of syringe plunger in vitro testing of eq [1] (*a*) Frequency shifts (Δf) from orifice jet (*b*) Pressure tracing ΔP_M = pressure gradient

tracing to allow for the left atrium to right heart catheter transmission time The area under the curve representing the gradient was determined by integration and divided by the elapsed diastolic time thus the parameter $\overline{\Delta P}$ (mean diastolic pressure gradient) was obtained *T* (diastolic filling time/min) (sec) was obtained by measuring diastolic and whole beat durations on the pressure tracings and then performing the appropriate calculations Generally 3–4 consecutive beats were used for the above determinations A_e (geometric orifice area) was then calculated from eq [8] using the cardiac output as determined with the direct Fick method as *Q* (mitral flow/min)

The curve representing V (orifice jet velocity) was constructed from the ultrasound data using $\cos\theta = 1$ in the doppler equation *V* (mean diastolic jet velocity) was obtained by integrating the constructed curve and dividing the integral by the elapsed diastolic time Again 3–4 consecutive beats were generally used for the determinations A_v (effective orifice area) was then calculated from eq [5] using the same *Q* and *T* as were used in the determination of A_e

RESULTS

In vitro and in vivo validity of eq [1]

Fig 3 presents a representative frequency analysis of the frequency shifts from the orifice jet and the concomitant pressure gradient obtained during a single depression of the syringe plunger The course of the maximum frequency shift is represented by a curve enveloping the shaded area (Fig 3*a*) the amount of blackening within the shaded area is related to the energy in the reflected ultrasonic beam The pressure tracing from the single transducer (Fig 3*b*) represents the pressure gradient across the orifice because the area of the fluid surface in the reservoir was so large that the fluid level changed negligibly during a single depression of the syringe plunger Fig 4 presents a comparison between the pressure gradient measured by the pressure transducer system (ΔP_M) and the gradient calculated from the ultrasound data (ΔP_U) In Fig 4 only the point of peak pressure gradient and peak frequency shift has been used for each plunger depression For the 8 mm diameter orifice pressure gradients of 3–108 mmHg were obtained and the ratio $\Delta P_U/\Delta P_M$ was close to 1 throughout this range of gradients For the 3.5 and 1.5 mm diameter orifices the ratio $\Delta P_U/\Delta P_M$ increases as the pressure gradient increases For the 3.5 mm diameter orifice the ratio reaches un...

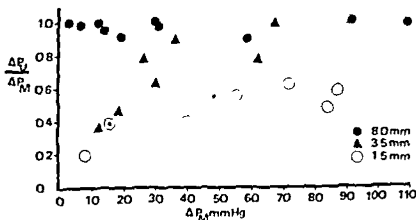


Fig 4 Results of in vitro tests of eq [1] A comparison of the gradient as calculated from the ultrasound data ΔP_U and the gradient determined with manometry ΔP_M

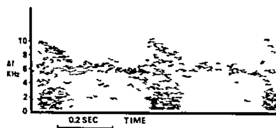


Fig 5 Frequency shifts (Δf) from orifice jet in dog s abdominal aorta

about 67 mmHg but for the 1 5 mm diameter orifice the ratio is still only about 0 6 at a gradient of 87 mmHg

Fig 5 presents a representative frequency analysis of the frequency shifts from the orifice implanted in a dog s abdominal aorta In order to avoid errors due to the effects of the frequency response of the catheter-pressure transducer system ΔP_U and ΔP_M were compared only at the end of diastole where relatively steady state conditions prevailed At this point in the cardiac cycle the pressure above the orifice was 90 5 mmHg and below the orifice 59 mmHg whereas the concomitant maximum frequency shift was 6 5 KHz Thus at a pressure gradient of 31 5 mmHg the ratio $\Delta P_L / \Delta P_M$ was calculated to be 0 64 for the 1 mm diameter orifice

Patient studies

Frequency analyses of satisfactory quality were obtained from all patients studied Fig 6 presents a representative frequency analysis of consecutive beats in patient 2 In all patients the frequency shifts due to the opening and closing motion of the mitral leaflets were observed in the frequency analysis as dense triangular areas at the start and end of diastole T can thus be determined from the spacing of these triangular areas

The numerical results of the various parameters determined and the calculated orifice areas are presented in Table 1 $A_e/0\ 775$ was larger than A_* in all patients studied The mean difference between $A_e/0\ 775$ and A_* was 0 19 cm² and the linear correlation coefficient between the two 0 975

DISCUSSION

The results of the in vitro experiments (Fig 4) demonstrate the dependence of the validity of eq [1] upon the Reynolds number The largest orifice area used in the tests 0 5 cm² is somewhat smaller than those usually encountered in mitral stenosis and the viscosity of bank blood at room temperature can be expected to be larger than the viscosity of blood in vivo Thus in these respects the in vitro tests were performed at Reynolds numbers lower than those that can be expected in mitral stenosis On the other hand stenotic mitral orifices are often slit like and the length of the stenosis can exceed the 2 mm thickness of the orifices used these factors will tend to enhance viscous losses of energy Nevertheless the results indicate that it is reasonable to expect small and possibly negligible viscous losses of energy in mitral stenosis so that if the effects of unsteady flow and the changing geometry of the mitral orifice can be neglected eq [1] through eq [6] can also be expected to be at least approximately valid for the flow of blood through stenotic mitral orifices

The results of the in vivo test of eq [1] where a 1 mm diameter orifice was implanted in a dog s abdominal aorta may demonstrate the effect of the in vivo viscosity of blood upon the ratio $\Delta P_L / \Delta P_M$ as the obtained ratio of 0 64 at a gradient of 31 5 mmHg is higher than the ratio predicted by the in vitro results (Fig 4) The in vivo test also demonstrates that one cannot expect eq [1] to be valid in stenosis of middle sized human arteries such as the common carotid and femoral arteries

The theoretical considerations demonstrate that A_* as determined from eq [7] or eq [8] does not

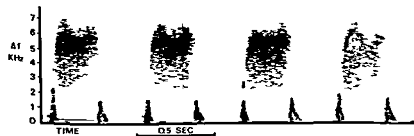


Fig 6 Frequency shifts (Δf) from mitral orifice in a patient with mitral stenosis Note representation of mitral leaflet motion

Table I Numerical results of patient studies

SR = sinus rhythm AF = atrial fibrillation $\dot{Q}$ = cardiac output (l/min) T = diastolic filling time/min (sec) $\overline{\Delta P}$ = mean diastolic gradient (mmHg) $\bar{V}$ = mean diastolic jet velocity (cm/sec) A_e = effective orifice area as determined from ultrasound data (cm²) A_g = geometric orifice area as determined from pressure tracings (cm²)

Pat no	Age (y)	Sex	Rhythm	$\dot{Q}$	T	$\overline{\Delta P}$	$\bar{V}$	A_e	$A_e/0.775$	A_g
1	57	♀	SR	4.2	26.5	5.2	114	1.39	1.79	[illegible]
2	66	♂	SR	6.5	30.0	19.9	218	0.99	1.27	[illegible]
3	52	♀	SR	4.9	33.6	7.2	118	1.23	1.59	1.36
4	57	♀	AF	5.6	25.2	3.5	77	2.88	3.72	2.97
5	51	♂	AF	8.1	24.0	6.3	129	2.61	3.37	3.36
6	24	♀	AF	4.9	23.0	7.4	133	1.60	2.06	1.96
7	71	♀	AF	3.1	40.5	9.5	130	0.59	0.76	0.62
8	58	♀	AF	1.8	23.1	7.4	131	0.60	0.77	[illegible]
9	47	♂	AF	4.7	28.8	6.6	107	1.52	1.96	1.55
10	55	♀	AF	5.5	25.7	8.0	139	1.54	1.99	1.89

represent the actual geometric orifice area but is directly proportional to the effective orifice area It should also be noted that the term $\sqrt{\overline{\Delta P}}$ (eq [6]) is theoretically correct and that the term $\overline{\sqrt{\Delta P}}$ (eq [8]) is an approximation Mathematically it can be *shown that the difference between these two terms* is negligible when the mitral pressure gradient is large as in Gorlin and Gorlin s patient material (1) *but when the gradient is smaller as after commi*surotomy and in prosthetic mitral valves the difference between the two terms assumes a significant magnitude It therefore seems appropriate to use A_e as determined from eq [5] or eq [6] for the quantification of mitral flow obstruction

Under ideal test conditions i e with an ultra[illegible] probe position that results in $\cos\theta = 1$ in the [illegible]ler equation with a pulmonary artery wedge [illegible]sure that is identical to the left atrial pressure with a pressure gradient that is such that $\sqrt{\overline{\Delta P}} = \overline{\sqrt{\Delta P}}$ and with negligible losses of viscous energy in the mitral orifice the terms $A_e/0.775$ and A_g will be identical Deviations from this ideal situation will however cause the values of the two terms to diverge so that $A_e/0.775 > A_g$ Thus failure to achieve $\cos\theta = 1$ will result in $\bar{V}$ being less than the actual value and effect overestimation of A_e Similarly dilution of the pulmonary artery wedge pressure by the pulmonary artery pressure frictional losses in the flow channels between the right heart catheter and the left atrium and damping of pressure expansion waves en route from the left atrium to the right heart catheter (3) will all tend to overestimate $\overline{\Delta P}$ and thus effect underestimation of A_g If there is a significant difference between the terms $\sqrt{\overline{\Delta P}}$ and $\overline{\sqrt{\Delta P}}$ it can be shown mathematically that the latter term will be the larger thus effecting underestimation of A_g Finally inspection of eq [5] and eq [8] will demonstrate that significant viscous losses in the mitral orifice will effect $A_e/0.775 > A_g$ The finding that $A_e/0.775$ is consistently larger than A_g in the 10 patients studied is therefore practically predictable on theoretical grounds

On the basis of this investigation it seems reasonable to conclude that the presented ultrasound method represents an alternative to the conventional catheterization methods used for quantification of mitral flow obstruction The ultrasound method has the advantage that invasive procedures are necessary only to the extent that they are needed for the determination of the mitral flow rate

ACKNOWLEDGEMENT

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Restricted Lignocaine Prophylaxis in Acute Myocardial Infarction

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ABSTRACT In a consecutive series of 274 AMI cases ventricular tachycardia (VT), defined as three or more ventricular premature beats (VPBs) in succession but not VPBs, has been used as the indication for ventricular fibrillation (VF) prophylaxis No primary VF occurred and this fits with the hypothesis of VT as a sufficient indication for prophylaxis against primary VF Six patients developed complicating VF (preceded by rales or hypotension but not frank pulmonary edema or shock) Four of the six patients (67%) had VT (0–1 5 hours) before VF, while the mean VT incidence of the six corresponding monitoring periods in 247 non VF patients was 5% Three of the four VT patients were on lignocaine/procainamide when VF developed Thus VT is acceptable as the only warning arrhythmia even in complicating VF but antiarrhythmic drugs do not seem to have the same prophylactic efficacy in complicating VF as in primary VF Another 21 patients developed VF *during* shock frank pulmonary edema or manipulating a pacemaker catheter within the heart

The main objective of the coronary care units (CCU) is commonly stated as reducing the number of deaths from primary ventricular fibrillation (PVF) in acute myocardial infarction (AMI) preferably by prophylactic antiarrhythmic treatment of so-called warning arrhythmias However as patients with complicating VF (distinguished from agonal VF) have a hospital mortality far from 100% they should be included in the aim which could better be formulated as prophylaxis against non agonal VF

The PVF warning arrhythmias include frequent multiform paired or early ventricular premature beats (VPB) as well as ventricular tachycardia (VT) (5) However when recently studied by continuous ECG recording or tape 25–40% of the primary VFs were not preceded by any warning arrhythmias (1 4) moreover the premonitory arrhythmias occurred as frequently in patients with primary VF as in those without (4) Although this must raise doubts about at least most of the premonitory arrhythmias i e the VPBs VT (of one or another duration) could still be a warning and this is possibly also valid for complicating VF Therefore since 1972 VPBs have not been an indication of antiarrhythmic treatment in our CCU but VT and VF still are Here the resulting VT and VF incidences in the first two years will be reported

MATERIAL AND METHODS

AMI was diagnosed in 274 cases (248 patients) in the CCU at Huddinge Hospital from Sept 1972 to Oct 1974 The criteria for the diagnosis were apart from the subjective symptoms leading to the suspicion the development of pathological Q waves R progression or localized ST elevation *or* two or more elevated S-GOT values with a maximum at about 24 hours after onset of symptoms and greater than a S-GPT maximum *or* myocardial necrosis at autopsy corresponding in age to the onset of symptoms

The mean age of the patients was 65 years 41% of them were admitted within three hours after onset of symptoms The mean S-GOT maximum was 165 U (upper normal limit 40 U) The hospital mortality was 20%

The CCU stay lasted 69 hours as an average During this period the ECG was observed continuously by the nurses on a central memory oscilloscope and they documented their arrhythmia diagnoses by cutting out strips from the continuous recording of the monitored ECG The strips were reexamined at the next round VT was defined as three or more VPBs in succession

During the CCU stay the lungs were auscultated for rales at least four times a day and more than a few scattered basal rales lead to the diagnosis of left heart failure BP was recorded at least every hour and hypotension was

Table 1 *Data on the six patients with complicating ventricular fibrillation and the 247 patients without ventricular fibrillation*

Case no	Left heart failure/hypotension before VF	Hospital outcome	Hours between VT and VF	Hours in CCU at VF	No of non VF patients with VT during as many hours in CCU
1	Basal rales	Survival	No VT	0	0
2	Rales up to scapula	Death	VT just before VF	0 5	2
3	Basal rales	Death	1	3	6
4	Rales up to scapula	Death	1 5	3 5	6
5	Hypotension	Death	1	5	10
6	Basal rales	Survival	No VT	13	45
					Mean 12 (5%)

defined as a controlled systolic recording of 90 mmHg or less

The CCU treatment followed conventional outlines except that VPBs did *not* indicate prophylactic antiarrhythmic treatment The only indications for lignocaine were VT and VF The lignocaine dose was 75 mg i v followed *by 2 mg/min as an i v infusion A first recurrence of VT* indicated another 75 mg i v All other relapses of VT or *VF prompted the addition of procainamide to the continued* lignocaine treatment

RESULTS

Twenty seven patients (10%) developed one or more attacks of VF *during* the CCU stay No VF was primary and six cases were classified as complicating because of heart failure or hypotension according to Lawrie et al (2) The incidence of heart failure and/or hypotension in the whole series was ??% The remaining 21 VFs occurred during shock pulmonary edema or manipulating a pacemaker catheter within the heart No primary VF in this series fits with the hypothesis that VF is a sufficient indication for prophylaxis against primary VF in AMI

Of the six patients with complicating VF five had mild or moderate left heart failure at the time of VF and one had hypotension (Table I) They were all men aged 40–76 years (mean 54) Their VFs occurred 5–29 hours (mean 15) after onset of symptoms They were admitted to the CCU with a delay of 2–24 hours (mean 11) which did not differ significantly from that of the rest of the series Nor did their mean S-GOT maxima differ from that of the remaining patients Two of the six patients survived the hospital stay and none died from the actual VF

VT occurred in 113 cases (41%) Four of the six patients (67%) with complicating VF developed VT before VF and in another one VF occurred immediately on arrival leaving no pre VF monitoring time The mean VT incidence of the six corresponding monitoring periods in the 247 non VF cases was 5% Thus VT was a significant VF warning arrhythmia against complicating VF ($p<0.05$)

In three of the four patients the VT occurred so long before VF (1–1 5 mean 1 1 hours) that a lignocaine prophylaxis could be started Because of recurrence of VT two of the three patients had also received 600–700 mg procainamide i v before the VF

DISCUSSION

In the present series of consecutive AMI cases VPBs were not considered to be warning arrhythmias and did not indicate antiarrhythmic treatment *VT was the only indication for VF prophylaxis* This restricted prophylaxis resulted in no primary VF which corresponds well to 0–4% primary VF in reported series where even VPBs were an indication for antiarrhythmic prophylaxis (2 3 6 7) This correspondence could not be attributed to higher age longer admission delay or shorter CCU stay in the present study

Complicating VF is about as common as primary VF and the hospital mortality of patients with complicating VF is far from the 100% of agonal VF (?) Thus prophylaxis against complicating VF is by no means unimportant provided that VT is a warning and antiarrhythmic drugs are effective in this setting too VT was found to be an acceptable warning but the value of the prophylaxis cannot be judged from this study It failed in three patients and it is not known in how many it was effective

The efficacy also depends upon the dosage used and the lignocaine dose in this series was probably somewhat low in circulatory uncomplicated patients below the age of 60 in comparison with the doses used by Lie et al (3)

The VT diagnoses were based on the nurses detection The nurses detect far from the whole incidence but until arrhythmia detectors are common in patient care the nurses detection rate and not the true incidence is the important figure

The VT incidence during the CCU stay 41% corresponds well to the figure of 40% found in another Stockholm hospital using the same monitoring routine and VT criteria (7) In the non treated series of Lie et al (4) the incidence was 5% This great difference in VT incidence probably reflects different VT criteria

ACKNOWLEDGEMENT

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Haemodynamic Effects of Four Months' Mefruside Therapy in Hypertensive Patients

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ABSTRACT The haemodynamic changes after 4 months mefruside therapy in 13 patients with essential hypertension have been studied Intraarterial BP was significantly reduced both at rest supine and during standardized leg exercise in sitting position The reduction was caused mainly by a decrease in cardiac output in about half of the patients and mainly by a decrease in total peripheral vascular resistance in the remainder Thus, for the total material there was no significant change in either cardiac output or total peripheral vascular resistance At rest, however, there was a significant decrease in stroke volume ($p<0.05$) and an increase in heart rate ($p<0.05$) On changing from supine to sitting position the average systolic and diastolic pressures increased before and decreased after therapy the differences being significant The results indicate that the hypotensive effect of long term saluretic therapy is accomplished by a decrease in cardiac output and/or peripheral vascular resistance, with large interindividual variations

Saluretics are well established in the treatment of hypertension The hypotensive effect of such therapy is well documented but the mechanisms involved are still poorly known Conway and Lauwers (6) showed in a study on 17 patients that the initial BP reduction during treatment with chlorothiazide was caused by a decreased cardiac output but that the reduction after one month of treatment was due to a decrease in the total peripheral resistance Lund Johansen (15) demonstrated that chlorthalidone and hydrochlorothiazide caused comparable BP reductions after one year of therapy elicited mainly by a reduced cardiac output for chlorthalidone and mainly by a reduction of the total peripheral resistance for hydrochlorothiazide

Because of these controversial reports about the haemodynamic effects of long term saluretic treatment it was considered of interest to study these mechanisms further

The saluretic used was mefruside Bergstrom et al (3) have shown that it is an effective antihypertensive drug and that the intracellular potassium content in muscle is unchanged but the intracellular water content reduced after 5 months of antihypertensive therapy without potassium substitution Mefruside belongs to the sulfonamide group and its chemical structure resembles thiazide diuretics and furosemide The saluretic effect is caused by an inhibition of sodium and water reabsorption in the cortical part of Henle s loop (17) After peroral administration mefruside is absorbed quickly and completely in the intestine Given in a dose of 50 mg per os the effect on electrolyte and water excretion was still optimal after 12 hours (2)

MATERIAL

Thirteen non hospitalized patients (8 men and 5 women) with essential hypertension were included in the study (Table I) 6 belonged to group I and 7 to group II according to the WHO classification Their mean age was 48 years (range 39–57) No patient had received hypotensive therapy before the study Patients with secondary hypertension and other diseases were excluded on the basis of routine clinical examinations including radiorenogram and i v urography

METHODS

Before treatment intraarterial BP (arteria brachialis) cardiac output (dye dilution technique) heart rate (ECG registration) and oxygen consumption (Douglas bag technique) were measured in supine position in sitting position on a bicycle ergometer (6–9 min) and during a

Table I *Some anthropometric and clinical data on the patients*

Pat no	Sex	Age (y)	Height (cm)	Weight (kg)	BSA (m^2)	BP[a] (mmHg)	Hypertensive stage[b]
1	♀	55	158	55	1 58	205/115	II
2	♀	57	161	51	1 52	180/105	I
3	♂	40	182	79	2 00	180/115	I
4	♀	49	166	75	1 83	185/110	I
5	♂	41	176	69	1 84	180/120	II
6	♂	54	177	73	1 89	205/115	II
7	♀	51	157	49	1 47	205/120	II
8	♂	42	179	88	2 07	175/125	II
9	♂	44	176	80	1 96	185/110	II
10	♂	56	180	75	1 94	170/110	I
11	♂	43	170	55	1 63	170/110	I
12	♂	50	183	89	2 12	215/135	I
13	♀	39	154	66	1 64	210/120	II
$\bar{x}$		47 8	170 7	69 3	1 80	189 6/116 2	
±S E M		±1 81	±2 85	±3 75	±0 06	±4 44±2 20	

[a] Initial non invasive values after 5 min rest in the recumbent position
[b] Criteria proposed by the WHO in 1962

standardized leg exercise (men 300–600–900 kpm/min women 200–400–600 kpm/min) The following variables were also determined total Hb and blood volume according to the alveolar CO method (19) and serum potassium serum sodium serum bicarbonate uric acid and sodium and potassium excretion in urine with routine clinical methods After four months of mefruside therapy 25 mg/day all these investigations were repeated

The significance of the difference between mean values before and after treatment has been evaluated with Student s *t* test

RESULTS

supine (Table II) The mean oxygen uptake unchanged before treatment 272 and after 274 ml/min The intraarterial BP de*creased significantly from 189/105 to 166/97* mmHg Heart rate increased significantly from 65 to 69 beats/min and the stroke volume decreased significantly from 82 to 72 ml Cardiac output and total peripheral resistance showed slight mean decreases which failed to reach significance on account of the large interindividual variations (Fig 1)

Rest sitting Before treatment the change from lying to sitting position on the bicycle ergometer resulted in a slight but non significant increase in systolic and diastolic BP (Fig 2) After four months of mefruside therapy *the average values of these* pressures decreased at this change but not significantly However for both pressures the difference *in reaction before and after therapy* was significant ($p<0.05$) The changes in stroke volume cardiac output heart rate and peripheral vascular re*sistance on changing from supine to sitting position* were not significantly different before and after therapy

Exercise The haemodynamic variables have been compared only at work load II (men 600 kpm/min and women 400 kpm/min) considered to be a moderate load Oxygen uptake was the same after treatment as before (means 1452 and 1454 ml/min respectively) The intraarterial blood pressure decreased significantly *from 225/113 to* 214/105 mmHg Heart rate stroke volume cardiac

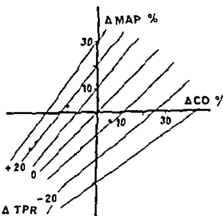


Fig 1 Individual percentage changes in mean arterial BP related to the individual percentage changes in cardiac output following 4 months mefruside therapy The diagonal lines indicate the percentage changes in the calculated total peripheral vascular resistance

Table II *Some haemodynamic parameters before (C) and after (M) 4 months treatment with mefruside 25 mg/day at rest in the recumbent position and during exercise in the sitting position (mean±S E M)*
d=difference p=level of significance

	BP (mmHg)		Heart rate	Cardiac output	Stroke volume	Total peripheral vascular resistance
	Systolic	Diastolic	(beats/min)	(l/min)	(ml)	(U)
Rest						
C	189±4 9	105±2 4	65±1 6	5 3±0 30	82±4 7	26 5±1 40
M	166±3 7	97±3 1	69±2 9	4 9±0 22	72±4 2	26 1±1 39
d	−23±3 6	−8±1 6	4±1 9	−0 4±0 25	−10±4 4	−0 4±0 12
p	<0 001	<0 001	<0 05	>0 1	<0 05	>0 1
Work load II						
C	225±8 8	113±4 7	133±4 4	11 8±0 46	90±4 41	13 7±1 09
M	214±7 2	105±4 6	132±5 9	10 9±0 53	86±5 5	13 7±1 06
d	−11±4 1	−8±3 2	−1±4 0	−0 9±0 45	−4±3 8	0 ±0 61
p	<0 025	<0 01	>0 1	<0 1	>0 1	>0 1

output and total peripheral vascular resistance were not significantly changed after therapy (Table II)

From rest to exercise the intraarterial systolic and diastolic BP increased by a mean of 36/8 mmHg before treatment and 48/8 mmHg after treatment (Fig 2) The increase in systolic pressure after therapy was significantly steeper than before ($p<0.025$) The increases in heart rate stroke volume and cardiac output and the decrease in total peripheral resistance did not differ significantly before and after therapy

Other results

Total Hb decreased significantly after treatment from 741±56 to 665±47 g ($p<0.05$) Blood volume was not significantly changed (5 1±0 3 and 4 9±0 3 l respectively) neither was hematocrit (41±0 6 and 41±0 8 mg/100 ml) Serum potassium decreased significantly from 4 0±0 1 to 3 6±0 1 mEq/l ($p<0.05$) and standard bicarbonate increased significantly from 24±0 3 to 25±0 4 mEq/l ($p<0.05$) Uric acid increased significantly from 4 1±0 4 to 5 9±0 4 mg/100 ml ($p<0.005$) There was no significant change either in serum sodium or in the urinary excretion of sodium and potassium

DISCUSSION

Our material consists of both men and women with about equal numbers in WHO groups I and II judging from the materials of Sannerstedt (18) and Lund Johansen (14) this distribution seems to be rather representative for middle age patients with light to moderate essential hypertension Their mean cardiac output was within the normal range and consequently the calculated total peripheral resistance was increased The reduction of BP after 4 months of mefruside therapy was of the same order as in earlier investigations (2 3 13 16) This effect is also similar to that of other types of diuretics chlorothiazide chlorthalidone polythiazide hydrochlorothiazide and spironolactone (1 4 14)

The reduction of BP was related to a slight mean decrease in both cardiac output and the calculated

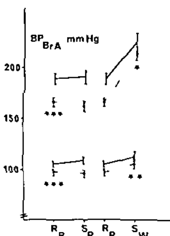


Fig 2 Systolic and diastolic BP at rest in the recumbent (R_R) and in the sitting (S_R) position and during work (S_W) before (—) and after (O---O) 4 months mefruside therapy (mean±S E M) The levels of significance are given for differences between means at rest and during work

total peripheral resistance though neither change was significant In about half of the patients the reduction of BP mainly reflected a decreased cardiac output in the other half a decreased total peripheral resistance (Fig 1) These results are not in agreement with Conway and Lauwers (6) who after one month of chlorothiazide treatment found a slightly increased cardiac index and a significantly reduced total peripheral resistance Only initially, after 1–2 weeks, did they find an increased total peripheral resistance and a decreased cardiac out put

Lund Johansen (15) studying 15 patients treated for one year with hydrochlorothiazide found an unchanged cardiac index and a significantly de creased total peripheral resistance On the other hand, in a somewhat older group of 9 patients with hypertension of longer duration he showed a more pronounced decrease in the cardiac index and an unchanged total peripheral resistance after treat ment with chlorthalidone In the present material however the percentage decrease in cardiac output after treatment did not correlate with the age Neither were there any correlations between this decrease and initial arterial BP cardiac output or total peripheral resistance There was however a tendency for the decrease in cardiac output to be more marked in the patients belonging to WHO group II than in those of group I This may indicate a tendency to a more marked decrease in cardiac output in patients with hypertension of longer dura tion in agreement with the findings of Lund hansen

Saluretic therapy induces an initial decrease in plasma volume (6 7 9 21) which may cause a decrease in stroke volume and cardiac output Hansen (12), Tarazi et al (20) and Castenfors et al (5) showed that blood or plasma volume was de creased even after prolonged diuretic treatment In our material however the blood volume did not change significantly though the total Hb was significantly decreased which could indicate a re duced intravascular volume and contribute to the significant decrease in stroke volume However the change in blood volume after therapy was not related to the change in BP or cardiac output Thus changes in blood volume probably do not play a major part in the decreased cardiac output or stroke volume after long term saluretic treatment This is in agreement with the findings of Finnerty et al (11) and of Davidov et al (8) who showed that it was not the decreased plasma volume but the decreased extracellular volume which seemed to contribute to the decreased BP after furosemide administration in hypertensive patients volume re expansion with isotonic glucose infusion restored BP to the pre treatment level in spite of a more markedly negative sodium balance and a persistently decreased plasma volume

On changing from lying to upright position the reaction of the systolic and diastolic pressures after mefruside treatment was significantly different from before (Fig 2) This changed orthostatic BP reac tion was not related to any change in the orthostatic reaction of cardiac output stroke volume or heart rate or to any change in blood volume following therapy The increase in calculated total peripheral resistance on changing from supine to upright posi tion was somewhat less marked after therapy (+4 4 U) than before (+5 7 U) This might be related to a smaller vasoconstrictor response to increased sympathetic tonus and agrees with the finding o Fine et al (10) who reported a decreased pressor response to noradrenaline injection after saluretic therapy They also showed a decreased cate cholamine excretion in upright position after saluretic therapy in essential hypertension This may be one explanation for the changed orthostatic BP reaction in our material after saluretic treatment

During a standardized leg exercise on bicycle ergometer in sitting position BP was significantly lower after than before treatment but the decrease was not so marked as at rest in supine position This corresponds to a significantly steeper increase in systolic BP during exercise after saluretic treatment than before and may be related to organic structural changes in the peripheral arterial vascular bed which did not seem to be influenced by 4 months of saluretic therapy This agrees with the almost identical mean calculated total peripheral resistance before and after therapy at a standardized work load (Table II)

In conclusion in this material saluretic therapy elicited a reduction of BP through a decrease in both cardiac output and total peripheral resistance the effect being maximal in supine position and less marked during exercise

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Haemodynamic Effects of Saluretic Treatment and β-receptor Blockade in Patients with Essential Hypertension

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ABSTRACT The long term haemodynamic effects of treatment with mefruside alone or in combination with alprenolol have been studied in nine patients with essential hypertension After four months of mefruside therapy alone there was a significant decrease in intraarterial BP both at rest and during standardized leg exercise Cardiac output and calculated total peripheral vascular resistance (TPVR) showed only minor mean decreases which were not statistically significant Stroke volume tended to decrease and heart rate to increase After another four months' treatment with addition of alprenolol there was a further significant decrease in intraarterial BP, related mainly to a further mean decrease in cardiac output which was not statistically significant Heart rate decreased significantly stroke volume was not significantly changed Compared with the pretreatment levels combination therapy induced a substantial decrease in intraarterial BP and a significant decrease in cardiac output both at rest in supine position and during standardized leg exercise TPVR decreased slightly both at rest and during exercise but the change was not statistically significant The results suggest that the decrease in BP after combined therapy with mefruside and alprenolol is mainly related to a decrease in cardiac output the changes in TPVR being not significant The additive hypotensive effect of alprenolol seems to be related in part to blocking of the increase in sympathetic activity that was found after treatment with mefruside alone

Treatment with saluretic and β adrenergic blocking agents is well established and efficient in controlling BP in hypertensive patients (8 11 20) but little is known about the haemodynamic effects of these drugs after long term therapy Conway and Lauwers (7) found a reduction of the total peripheral vascular resistance (TPVR) with no significant change in cardiac output after long term saluretic treatment On the other hand Lund Johansen (16) demonstrated a decreased cardiac output after long term treatment with chlorthalidone

In acute and short term studies β receptor blockade results mainly in a decrease in cardiac output with increased or unchanged TPVR (14 22 23) Following long term therapy TPVR may remain increased (17) or return to the pretreatment level (13) Combined therapy with a diuretic and a β receptor blocking agent is also effective in controlling BP in hypertensive patients (5) but no haemodynamic studies on this combination therapy appear to have been reported

The aim of the present study was to investigate the haemodynamic effects of long term treatment with a saluretic alone and in combination with a β blocking agent The saluretic agent used was mefruside (Baycaron®) for further details see Bevegård et al (4) and the β adrenergic blocking agent alprenolol (Aptin®) which in both animal and human experiments has also been shown to be a weak β receptor stimulator (1) Its ability to lower BP in hypertensive patients is well documented (3 9 12 24)

MATERIAL AND METHODS

The study was undertaken in nine untreated ambulatory patients with essential hypertension (5 men and 4 women) with a mean age of 48 years (range 40–55) Secondary hypertension was disregarded after routine clinical examination in all cases Six patients belonged to group I and 3 to group II according to the WHO classif

Table I *Some anthropometric and clinical data on the patients*

Pat no	Sex	Age (y)	BSA (m^2)	BP[a] (mmHg)	Hyper tensive stage[b]	Blood volume (l)	Work intensity at pulse rate of 170/min (kpm/min)
1	♀	49	1 83	185/110	I	5 1	650
2	♀	55	1 58	205/115	I	4 0	700
3	♀	40	1 62	245/140	I	5 4	500
4	♀	51	1 47	205/120	I	4 0	900
5	♂	50	2 12	215/135	I	8 8	1 400
6	♂	42	2 07	175/125	II	5 8	750
7	♂	54	1 89	205/115	II	4 7	550
8	♂	44	1 96	185/110	II	4 3	900
9	♂	43	1 63	170/110	I	5 3	1 000

[a] Initial non invasive values after 5 min rest recumbent
[b] Criteria proposed by the WHO in 1962

Anthropometric and clinical data are given in Table I These patients were selected from a larger group treated with mefruside for four months without attaining a sufficient decrease in BP (above 160/100 mmHg) and therefore given additional treatment with β adrenergic blockade The haemodynamic effects of saluretic treatment with mefruside alone in that group are reported separately (4)

Before treatment intraarterial BP heart rate and cardiac output were determined at rest supine as well as sitting on a bicycle ergometer (6–10 min) and also during upright leg exercise at standardized submaximal work loads Oxygen uptake was determined at rest in supine position and during exercise All investigations were performed in the morning The patients were then treated with 25 mg mefruside daily and four months later the haemodynamic investigation was repeated The patients then received additional treatment with alprenolol in individual doses of 0 4–1 2 g/day (mean 0 64) chosen so that the diastolic BP fell to below 100 mmHg After another four months of consecutive treatment with mefruside combined with alprenolol the haemodynamic investigations were repeated again The methods used for determination of haemodynamic variables and oxygen uptake are presented in detail in a separate article (4) TPVR and the total peripheral resistance index (*TPRI*) are calculated as the mean arterial BP divided by cardiac output and cardiac index respectively

Student s *t* test was used for evaluating the statistical significance of paired differences Mean values S E M and the level of significance are given

Table II *Effects of mefruside and alprenolol treatment on central haemodynamics at rest supine and during exercise in sitting position (mean ±S E M)*

O_2=oxygen uptake SAP=systolic intraarterial pressure DAP=diastolic intraarterial pressure HR=heart rate =stroke volume Q̇=cardiac output TPVR=total peripheral vascular resistance

/	I Before therapy	II Mefruside	III Mefruside+ alprenolol	Differences I-II		II-III		I-III	
Supine rest									
V_{O_2} (ml/min)	276±15	279±18	256±13	3	n s	−23	*	−20	*
SAP (mmHg)	200±8	174±7	154±5	−26	***	20	**	−46	***
DAP (mmHg)	109±4	102±4	90±3	−7	*	−12	**	−19	***
HR (beats/min)	72±4	77±4	67±3	5	n s	−10	***	−5	n s
SV (ml)	77±6	66±6	71±1	−11	n s	5	n s	−6	n s
Q̇ (l/min)	5 5±0 3	5 0±0 3	4 7±0 1	−0 5	n s	0 3	n s	−0 8	*
TPVR (U)	27 0±1 9	27 1±1 1	25 6±1 1	0 1	n s	−1 5	n s	1 4	n s
Sitting exercise									
V_{O_2} (ml/min)	1 375±81	1 488±101	1 417±94	113	n s	−71	n s	42	n s
SAP (mmHg)	238±16	220±10	188±11	−18	**	−32	**	−50	
DAP (mmHg)	114±7	104±5	94+5	−10	**	−10	*	−20	*
HR (beats/min)	134±6	135±8	113±4	1	n s	−22	**	21	
SV (ml)	85±8	79±8	85±4	−6	n s	6	n s	0	n s
Q̇ (l/min)	11 2±0 7	10 3±0 8	9 5±0 5	−0 9	n s	−0 8		1 7	
TPVR (U)	15 2±2 0	14 9±1 5	14 5±1 3	−0 3	n s	−0 4	n s	0 7	n s

* $p<0\ 05$ ** $p<0\ 01$ *** $p<0\ 001$ n s =non significant

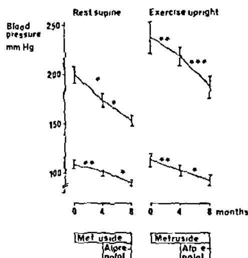


Fig 1 Systolic and diastolic intraarterial BP at rest and during work (men 600 kpm/min women 400 kpm/min) before and after treatment with mefruside alone and combined with alprenolol (mean ±S E M) * ** *** indicate *p* values of <0 05 <0 01 and <0 001 respectively for paired differences

RESULTS

Intraarterial blood pressure

Four months treatment with mefruside induced a significant decrease in intraarterial BP and the addition of alprenolol during another four months caused a further significant decrease The effects were similar at rest supine and during standardized leg exercise in sitting position (Table II Fig 1) The effect on systolic BP of mefruside treatment alone was somewhat more marked at rest than during sitting leg exercise following the addition of alprenolol this effect was more marked during exercise than at rest

Cardiac output and TPVR

Four months of mefruside treatment caused a minor but non significant decrease in cardiac output both at rest supine and during exercise After the additional treatment with alprenolol as well there was a further significant decrease in cardiac output during exercise but only a slight and non significant decrease at rest supine Compared with the untreated condition eight months of combination therapy induced a significant decrease in cardiac output both at rest supine and during exercise (Table II Fig 2) TPVR showed only small and insignificant changes both at rest supine and during exercise

Heart rate and stroke volume

After four months of mefruside treatment there was a mean decrease in stroke volume and an increase in heart rate which was not statistically significant After another four months on the combined therapy there was a significant decrease in heart rate and a slight mean increase in stroke volume which was not significant The changes were similar at rest supine and during exercise at standardized work loads (Table II)

Haemodynamic changes on transition from supine to upright position

Without therapy transition from supine to sitting position caused no significant changes in intraarterial pressures significant decreases in stroke volume and cardiac output and a significant increase in heart rate and TPVR

After four months of mefruside therapy diastolic BP reacted significantly differently on transition from supine to sitting position namely with a slight mean decrease instead of a slight mean increase (Table III) There were less marked decreases in stroke volume and cardiac output the differences being not statistically significant After another four months treatment with mefruside plus alprenolol the diastolic BP response returned to the pretreatment condition but differed significantly from that following treatment with mefruside alone

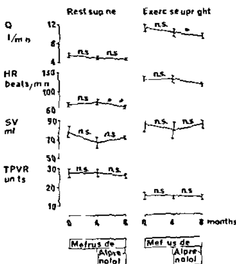


Fig 2 Cardiac output ($\dot{Q}$) heart rate (*HR*) stroke volume (*SV*) and calculated total peripheral vascular resistance (*TPVR*) at rest and during work before and after treatment with mefruside alone and combined with alprenolol n s =non significant

Table III *Changes in haemodynamic parameters on transition from supine to sitting position before and after therapy (mean ±1 S D)*
Abbreviations as in Table II

	I Before therapy	II Mef ruside	III Mefruside+ alprenolol	Significance of paired differences I–II	II–III	I–III
SAP (mmHg)	−1 1±14	−5 3±17	2 3±17	n s	n s	n s
DAP (mmHg)	2 8±10	−2 7±7	5 7±7	$p<0\ 05$	$p<0\ 01$	n s
MAP (mmHg)	−1 2±15	−6 4±10 9	4 3±12	n s	$p<0\ 025$	n s
HR (beats/min)	7 5±6 0	5 2±6 8	4 2±2 5	n s	n s	$p<0\ 05$
Q (l/min)	−1 2±0 8	−0 7±1 0	−0 7±1 1	n s	n s	n s
SV (ml)	−22±16	−14±15	−15±18	n s	n s	n s
TPVR (U)	6 9±2 9	4 3±4 1	7 3±6 6	n s	n s	n s

n s =non significant

On transition from supine to sitting position mean BP showed a mean decrease in the untreated condition and during treatment with mefruside alone but a mean increase when alprenolol was added which is a significantly different reaction compared with that caused by mefruside alone

DISCUSSION

This study confirms the usefulness of combining saluretic and β adrenergic blocking drugs in the treatment of essential hypertension (5 10 24) It has also been reported that combination therapy results in a better BP control in cases where either saluretic drugs or β receptor blockers alone prove unsatisfactory (6 18 19 21) This suggests the ibility of a synergistic effect of combination y which is supported by the finding of Ang all and Bystedt (2) that alprenolol combined with a saluretic drug reduces BP to the same extent as either of the drugs alone but in a dose of only ½

The haemodynamic effect of four months treatment with mefruside alone consisted of a significant decrease in intraarterial BP but no significant changes in the other haemodynamic variables This agrees with results from a somewhat larger material reported separately (4) though in supine position the latter also showed a significant decrease in stroke volume and a significant increase in heart rate possibly indicating an increased sympathetic activity in this condition During exercise there was again a significant decrease in intraarterial pressure but no significant changes in the other haemodynamic variables Thus this investigation does not support either the significant decrease in cardiac output (16) or the significant decrease in TPVR (7) that have been reported after long term saluretic treatment

Adding alprenolol to the saluretic treatment caused a further significant decrease in intraarterial BP At rest supine this effect was related to a small average decrease in both cardiac output and TPVR but neither change was statistically significant During exercise however the decrease in BP is probably explained mainly by a significant decrease in cardiac output The latter decrease was related to a significant decrease in heart rate both at rest and during exercise

It is important to note that although β adrenergic receptor blockade tended to decrease cardiac output in this material it increased the cardiac index in the patients in whom this index was lowest at rest supine or fell most after therapy with mefruside alone (Fig 3) A decreased cardiac index was found mainly in the patients in whom the index had been normal or increased which may suggest that they have a higher sympathetic activity The additional effect of β receptor blockade in lowering BP in these patients may be due to a counteraction of an increased sympathetic stimulation partly reflected by the increase in heart rate induced by mefruside alone The significant decrease in basal oxygen uptake during alprenolol treatment compared with the untreated condition as well as with the condition during treatment with mefruside alone also points to a decreased metabolic and possibly adrenergic activity after alprenolol treatment It may also be an indication that the patients with β adrenergic blockade at rest were in a more basal condition during the haemodynamic investigation

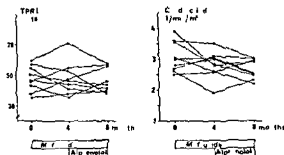


Fig 3 Effect of treatment on individual values for cardiac index and calculated total peripheral vascular resistance index (*TPRI*)

The haemodynamic changes at the transition from supine to sitting position were largely similar with and without treatment After four months treatment with mefruside alone however the decrease in BP was somewhat more marked This may indicate a decreased vasoconstrictor response and/or decreased reactivity of the resistance vessels to sympathetic stimuli after treatment with mefruside alone which agrees with the less marked mean increase in TPVR on transition from supine to sitting in this condition

The marked mean decrease in intraarterial BP (50/20 mmHg) after combined mefruside and alprenolol treatment compared with the untreated condition was mainly related to a significant decrease in cardiac output both at rest and during standardized exercise However TPVR did not differ significantly from the untreated condition due to a large interindividual variation (Fig 3) The four patients with the lowest TPRI in untreated condition all showed an increase after eight months of combination therapy while the five patients with the highest TPRI before treatment all showed a reduction This may suggest a decreased peripheral vasoconstriction and/or a diminished wall/lumen ratio in the resistance vessels (15 26) after treatment in the latter patients

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Right Atrial Monophasic Action Potential in Patients with Paroxysmal Supraventricular Tachyarrhythmias

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ABSTRACT Thirteen patients with paroxysmal atrial tachyarrhythmias (six atrial supraventricular tachycardia, six atrial fibrillation and one atrial flutter) have been investigated by recording of monophasic action potentials (MAP) during a period free from arrhythmias None of the patients differed from healthy individuals with respect to any of the MAP variables Occasional recordings showed values not found in healthy individuals such as prolonged duration of the MAP or slow phase 4 depolarization The atrial effective refractory period (AERP) was determined in two patients One AERP was short compared with values found in healthy individuals Thus some MAP recordings gave a possible explanation for the mechanism behind the arrhythmias, while other recordings showed no differences from results in healthy individuals

Monophasic action potentials (MAP) have previously been recorded during and immediately following atrial tachyarrhythmias (10 22) but the studies did not demonstrate any findings suggestive of the mechanism of the arrhythmias MAP recordings immediately after electrical conversion of atrial fibrillation however proved to be of prognostic value (9 25) with respect to relapse into atrial fibrillation

Six patients with supraventricular tachyarrhythmias were previously investigated by MAP recordings during a period free from arrhythmias (23) One recording showed a slow phase 4 depolarization and another a short duration of the MAP In the rest of the few investigations performed the MAP in this group of patients did not differ from the MAP of arrhythmia free subjects Since the number of patients in the above study was limited a further investigation has been performed in 13 patients with the same types of atrial dysrhythmias The aim was to evaluate further the MAP method with regard to probable arrhythmia provoking factors in this group of patients The findings were compared with MAP recordings from healthy individuals (4) The atrial effective refractory period (AERP) was also determined in two of the patients

MATERIAL

Thirteen patients (five females and eight males) aged 22–76 years participated Pertinent clinical data the frequency of the attacks and the duration of each attack are given in Table I All patients informed consent was obtained All suffered from some paroxysmal supraventricular tachyarrhythmia All patients had had their arrhythmias for between eight months and 25 years except one (no 11) who had suffered from paroxysmal atrial fibrillation for only one week but with several attacks each day In all patients the tachyarrhythmias had been recorded and diagnosed using ordinary ECG leads

Seven patients were on no drug treatment Two patients were on alprenolol in sustained release form (Aptin Duretter® Hassle) The medication was stopped during 24–36 hours before the investigation The patients on digoxin (Digoxin ACO) and quinidine (Kinidin Duretter® Hassle) received the last dose on the morning of the day before the investigation as did the patient on phenytoin (Difhydan® Leo) All had normal serum electrolytes (sodium potassium calcium chlorides phosphates bicarbonates) and normal blood levels of thyroxine and triiodothyronine on the day of the investigation They had no signs of thyroid dysfunction The MAP recordings were performed within seven days of the last episode of tachyarrhythmia

METHODS

MAP recording

All patients were examined at rest in the supine during fasting and without premedication T

Table I *Selected clinical data*

PSVT=paroxysmal supraventricular tachycardia PAF=paroxysmal atrial fibrillation PAFl=paroxysmal atrial flutter ASD=atrial septal defect IHD=ischemic heart disease, y=year m=month w=week d=day h=hour min=minutes

Case no	Age (y)	Sex	Arrhythmia	Additional cardio vascular disease	Heart volume (ml/m²)	Duration of arrhythmias	Each attack Maximal no	Each attack Maximal duration	Drug
1	62	♀	PSVT		590/340	12 y	1/y	5 h	
2	60	♀	PSVT	ASD	800/460	14 y	2/d	8 h	Digoxin Quinidine
3	22	♂	PSVT		640/330	11 y	1/m	2 h	Alprenolol
4	70	♂	PSVT		600/440	8 m	2/w	2 h	
5	68	♀	PSVT		650/370	2 y	4/y	1 h	
6	48	♂	PSVT		–	1 5 y	15/y	4 h	
7	59	♂	PAF		700/370	25 y	5/w	24 h	Digoxin Quinidine
8	76	♀	PAF	IHD	720/490	9 y	3/w	12 h	Phenytoin
9	54	♀	PAF		940/470	3 y	5/d	5 min	Digoxin Alprenolol
10	50	♂	PAF		790/420	1 y	3/y	3 h	
11	47	♂	PAF	IHD	1 040/510	1 w	5/d	3 min	
12	44	♂	PAF		890/440	10 y	2/d	2 min	Quinidine
13	61	♂	PAFl		730/350	2 y	2/m	3 h	

catheter was introduced percutaneously via the right femoral vein and advanced to the right atrium under fluoroscopic control The MAP signal was recorded together with a unipolar right atrial electrogram and a precordial ECG The signals were amplified with a DC recording system and recorded on an Ultralette writer as well as on a tape recorder Details of the technique for MAP recording and of the analysis of the single MAP have been described elsewhere (4) In the present work the MAP was analyzed with respect to amplitude duration at 50% and 90% repolarization and the relative repolarization rate during phase 3 (RRR ph 3) The durations at 50% 90% repolarization were transformed to a cycle length 800 msec according to the regression line between the duration of the MAP and the cycle length described previously (4) According to this formula a decrease in cycle length of 100 msec corresponds to a shortening of the duration of the MAP (90%) by 10 9 msec As the RRR ph 3 does not show any significant correlation to cycle length in healthy males no transformation according to cycle length was necessary (4) The intention was to obtain two MAP recordings in every individual but in three of the patients it was possible to perform only one recording for technical reasons

Atrial pacing and determination of the atrial effective refractory period

MAP was recorded during atrial pacing in one patient with paroxysmal supraventricular tachycardia (no 3) and in the patient with paroxysmal atrial flutter (no 13) The paced cycle length was reduced successively in steps of 100 msec from 800 to 400 msec The frequency dependence of the MAP variables was analyzed AERP was determined using the extra stimulus technique (28) with the pacemaker electrode close to the sinus node Only a small number of AERP investigations were performed as the technique was not established until near the end of this study

RESULTS

The results of the individual analyses of the MAP recordings are shown in Table II In the statistical analyses only the first recording in each patient was used as two recordings were not performed in all patients The cycle lengths and different MAP variables for these two groups of patients were comparable and the groups did not differ significantly from healthy males (4) (Fig 1)

The duration of the MAP at 50% repolarization in the individual patients was within ±2 S D of the mean value (104–210 msec) as judged from healthy males (Fig 1) The second recording in patient 11 however was 233 msec which is above +2 S D This patient exhibited a duration of the MAP at 90% repolarization which was above the mean value +2 S D found in healthy males (187–345 msec) The difference between 1st and 2nd recordings in this patient (284/400 msec) was great compared with the intraindividual variation in healthy males (4) The second recording in patient 13 with paroxysmal atrial flutter also gave a value (90%) above the mean value +2 S D and the difference between the two recordings was also rather great

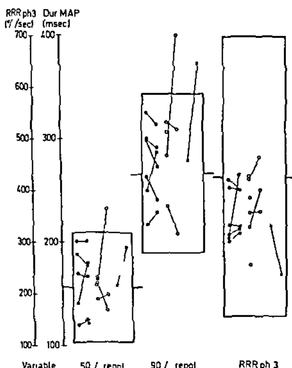


Fig 1 Individual values of the MAP variables (duration at 50% and 90% repolarization and RRR ph 3) for patients with PSVT (●) PAF (O) and PAFl (×) Thin lines indicate two recordings in the same patient The rectangles indicate mean ±2 S D for healthy males Abbreviations as in Table I

The RRR ph 3 was within the mean value ±2 S D of healthy males (154–696%/sec) for all patients and recordings

The MAP recordings for patient 1 showed a slow depolarization during phase 4 in one of the catheter positions (Fig 2)

The duration of MAP at 90% repolarization increased successively with increased paced cycle length in the two patients investigated (Table III)

AERP was determined in two patients (nos 6 and 9) The values were 164 and 304 msec at paced cycle lengths of 600 and 800 msec respectively Patient 6 had shorter AERP than healthy males (5) (mean ±2 S D =179–327 msec)

No harmful side-effects were seen in any of the patients in connection with the investigations No atrial tachyarrhythmias were provoked during atrial pacing with early ectopic beats

DISCUSSION

There are two theories concerning the electrophysiological mechanism behind cardiac tachyarrhythmias i e focal discharge and re entry phenomenon (8 14 17 27 32 35) Focal discharge means that latent pacemaker cells start an ectopic focus which takes over control of the electrical activity from the sinus node Focal discharge might depend upon changes in the latent pacemaker cells such as alteration of the resting membrane potential the threshold potential increase of the diastolic depolarization during phase 4 or shortening of the duration of the action potential These alterations might be brought about by such conditions as anoxia hypopotassemia or stretching of the heart muscle fibers (2 12 31)

Behind the re entry phenomenon there is a unidirectional block and a slow conduction of impulses (33 34) These conduction properties are predominantly found within the AV node (3 11 18) but are also seen in the sinus node region (13 21 29) as well as within the atrium or ventricle Re entry outside the AV conduction system requires great differences in conduction properties or refractoriness between closely adjacent areas of the atrium or ventricles (1 8 19 20) Local extracellu

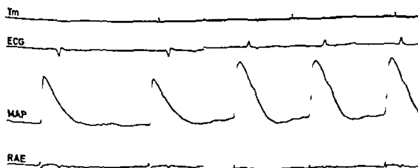


Fig 2 Right atrial MAP in one patient without (left) and one with (right) a slow phase 4 depolarization *RAE*=right atrial electrogram

Table II *Electrophysiological data from the right atrium during sinus rhythm*

Values transformed to cycle length 800 msec within parentheses

Case no	Cycle length (msec)	Amplitude (mV)	Duration of MAP (msec) 50% repol	90% repol	RRR ph 3 (%/sec)
1	704	7 0	135 (141)	240 (250)	404
	740	5 6	177(180)	280 (287)	400
2	898	5 1	195 (189)	311 (300)	308
	940	3 9	186 (178)	288 (273)	430
3	968	8 9	180 (170)	317 (299)	315
	956	9 5	176 (167)	309 (292)	325
4	838	6 0	135 (133)	267 (264)	333
	939	5 9	130 (122)	256 (241)	330
5	990	3 6	212 (201)	346 (325)	301
	1 100	3 5	218 (201)	347 (314)	316
6	763	5 1	118 (120)	213 (217)	421
	720	6 3	120 (125)	220 (229)	400
7	655	8 4	137 (145)	300 (316)	256
	710	6 6	142 (149)	299 (309)	259
8	1 110	2 3	179 (161)	269 (235)	421
	1 139	3 6	154 (134)	245 (208)	465
9	1 046	6 9	174 (160)	334 (307)	256
10	862	4 0	170 (166)	280 (273)	385
11	1 020	4 8	178 (165)	308 (284)	328
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13	802	7 0	158 (158)	279 (279)	333
	819	7 9	196 (195)	375 (373)	235

hypopotassemia might for instance give very v conduction In the clinical situation it is often impossible to classify arrhythmias with respect to focal discharge or re entry and in some arrhythmias both mechanisms are probably involved (30)

Neither the MAP nor the AERP can provide any information about the resting membrane potentials or the threshold potentials as arrhythmia provoking factors (6 16) A slow depolarization during phase 4 however might be detectable using the MAP recording technique In one of the present patients a slow phase 4 depolarization was detected This finding might indicate an atrial focal discharge mechanism behind the arrhythmia in this patient This phenomenon has never been observed in healthy males (4) and is probably not due to a technical failure The depolarization was rather slow This might be due to the fact that the MAP mirrors the electrical events in a certain number of cells under the suction electrode It is unlikely that more than a very limited number of these cells would show latent pacemaker cell characteristics The MAP signal is therefore probably composed of cells both with and without a phase 4 depolarization

A short duration of the MAP or AERP might be an arrhythmia provoking factor (8) In the present investigation no patient exhibited a short MAP duration compared with healthy males (4) contrary to findings in patients with supraventricular tachyarrhythmias in another study (23) One of the present patients (no 6) exhibited a short duration of the AERP compared with healthy males (mean ±S D =253±36 8 msec) (5)

A pronounced intraindividual difference in the duration of the MAP at 90% repolarization was found in two patients (nos 11 and 13) compared with healthy males (4) This phenomenon might mirror great differences in repolarization even between more adjacent areas of the atrium and might be an arrhythmia provoking factor This phenomenon has not been observed in healthy individuals On the other hand the very slow repolarization as judged from the long duration of the MAP in the same two patients might contribute to the origin of a re entry phenomenon (8)

The MAP and the AERP are influenced by other factors than those mentioned above such as the degree of vagal discharge electrolyte abnormalities (15) or altered thyroid state (7) The present patients were normal with respect to electrolytes and thyroid function The degree of vagal tone is difficult to evaluate but the heart rates were not especially low

Some patients were on drug treatment The duration of the MAP is not changed by alprenolol (24) The duration of the action potential as judged from

Table III *Duration of MAP and paced cycle length in one patient with PSVT (no 3) and one with PAFI (no 13)*

Abbreviations as in Table I

Case no	Repolarization %	Duration of MAP (msec) at paced cycle lengths of 400 msec	500 msec	600 msec	700 msec	800 msec
3	50	123	–	143	143	149
	90	250	–	269	290	300
13	50	162	180	188	197	198
	90	288	333	348	354	370

m croelectrode stud es ıs shortened by d goxın (15) and phenyto n (?6) and prolonged by quınıd ne (26) The drug effect might have changed the durat on of the MAP ın sp te of the treatment having been stopped for 24–36 hours The changes due to the different drugs are however probably small com pared with the wide l mits of normal values

Summarızing the results ıt ıs obvious that when regarded as a group the ınvestigated patients d d not exh b t consistent or ımmediately conclusive find ngs as to the mechan sm behind the arrhythmias as judged from determination of MAP Between the ep sodes of arrhythmias however some patients demonstrated MAP and AERP values that are not found ın healthy ındividuals and might be arrhyth mia provoking The negative results of the MAP record ng method during a period free from ar rhythm as are ın contrast with the prognostic value of the MAP duration after electroconversion of atrial f brillat on (10 25) On the other hand most supraventricular tachycard as are probably due to a re-entry phenomenon within the AV node (or sinus node) w th an electrophysiological pathology that cannot be detected with recordings ın the atrium

ACKNOWLEDGEMENTS

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Table III *Duration of MAP and paced cycle length in one patient with PSVT (no 3) and one with PAFl (no 13)*

Abbreviations as in Table I

Case no	Repolarization %	Duration of MAP (msec) at paced cycle lengths of 400 msec	500 msec	600 msec	700 msec	800 msec
3	50	123	-	143	143	149
	90	250	-	269	290	300
13	50	162	180	188	197	198
	90	288	333	348	354	370

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Human Atrial Conduction with Reference to Heart Rate and Refractory Periods

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ABSTRACT Atrial conduction time (ACT) between a stimulating pacemaker electrode and an "impulse-detecting" monophasic action potential (MAP) electrode has been determined in 20 healthy males A small increase in ACT was found with increasing paced heart rates Using the extra stimulus technique, ACT was found to increase when the early ectopics approached the atrial effective refractory period (AERP) The degree of ACT prolongation was similar at long and short basic ACTs indicating that the slowing of impulse propagation probably occurred near the simulating electrode and presumably within ½-1 cm from that electrode The increase in ACT close to the AERP was of importance when determining the atrioventricular refractory period In three recordings a supernormal phase of conduction was found The small errors of the MAP recordings in detecting the time of excitation make the method sensitive enough to detect small differences in ACT

Most investigators consider that the spread of impulses within the atrium occurs in a concentric fashion from the sinus node (7 11 17) though with a faster conduction velocity along preferential pathways within the atrium (9 14 15 16 18 22) The conduction of impulses within the dog atrium has been thoroughly invstigated (8) using the extra stimulus technique A pronounced increase was found in the conduction time of premature impulses delivered close to the atrial effective refractory period (AERP) and this increase was of importance in determining the atrioventricular effective refractory period (AVERP) A corresponding evaluation has not been performed in man In addition in 50% of the investigated hearts a supernormal phase of conduction was demonstrated at the end of the period of slow conduction In humans Agha et al (1) demonstrated supernormal atrial conduction in five of 18 patients If one closely inspects the figures in this article it is obvious that the conduction time increased close to the AERP this phenomenon however was not especially considered in that investigation In both the above studies (1 8) pacemaker electrodes situated near the AV node were used to detect the A wave of the right atrial electrogram It is often difficult however to exactly determine the onset of a premature atrial depolarization with this technique In the present study we have used the onset of phase 0 of the right atrial monophasic action potential (MAP) as a reliable index of the time of arrival of excitation at the electrode (13)

The present study is a "spin off" of an investigation concerning the AERP and the MAP of the right atrium (2 3) and illustrates supernormal and subnormal atrial conduction in healthy males and its role in the determination of AVERP In addition the influence of heart rate on atrial conduction time was evaluated

MATERIAL AND METHODS

Twenty male volunteers aged 25-60 years participated in the study They were all healthy as judged from clinical history physical examination ECG and ches X ray None had any electrolyte abnormalities They were all participants in a previous study concerning right atrial MAP and AERP (2 3)

Atrial conduction and heart rate

In nine subjects atrial pacing was performed close to the sinus node The cycle length was reduced from 800 to 400 msec in successive steps of 100 msec with pacing for about 30 sec at each frequency The MAP was simultaneously

Table III *ACT (msec) at different coupling intervals (S_1–S_2)*

The coupling intervals are calculated with the individual AERPs as zero point (ACT_b=ACT of basic cycle length ACT_e=ACT of the ectopics) *=the shortest S_1–S_2 interval conducted to the ventricles

Case no	ACT_b	ACT_e at (S_1–S_2)–individual AERPs 2	4	6	8	10–14	15–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	90–99	100–124	125–149	150–199	200–300
11	45	116*	–	112	–	–	100	92	–	–	–	–	–	–	–	–	44	–	–
18	13	49	–	–	–	51	–	–	–	–	–	19	16	16	14	14	–	–	13*
19	*20*	*26**	*21*	–	–	*20*	–	*21*	*20*	–	–	–	–	–	–	–	–	–	–
20	50	–	–	–	–	56	–	52	48	–	46	54*	–	–	–	51	–	–	–
21a	21	68*	–	65	64	63	–	50	42	–	–	–	–	–	–	–	22	–	[illegible]
21b	20	76*	–	–	–	–	–	–	–	–	36	–	–	–	–	–	–	20	–
22a	*51*	*104*	*104*	*104*	*103*	*104*	–	*98*	–	*84*	*60*	*54*	–	–	–	–	–	*52*	*52*
22b	52	96	–	96	96	92	–	84	–	–	–	–	56	–	–	–	–	–	–
23a	60	90	91	–	–	–	–	–	–	–	61	–	–	–	–	–	–	61	62
23b	60	95	89	89	89	93	–	–	–	–	–	61	–	–	–	–	–	61	–
25	30	–	76	76	–	–	67	60	51	40	38	33	–	–	–	–	–	30	29
26	44	–	100	92	100	92	–	–	–	–	–	51	–	–	–	44	–	–	44
28	57	91	89	87	84	84*	–	83	64	57	–	–	–	–	–	–	–	–	57
30a	65	–	100	103	–	–	95	90	82	–	–	–	–	–	–	–	66	–	–
30b	64	112	–	–	–	89	–	76	–	70	–	–	–	–	–	–	66	–	–
33	52	60*	–	57	–	–	–	48	52	–	–	–	–	52	–	–	–	–	–
38	81	134	130	129	128	129	–	120	112	104	97	89	83	80	81	80	–	–	–
40	52	74*	–	–	–	62	–	56	52	52	48	48	48	48	–	–	–	49	52

the refractory period and lasted until AERP+30 to about AERP+200 msec The prolongation of the ACT close to the AERP was less pronounced in these cases It is impossible to judge if supernormal conduction may have existed in some of the other recordings not shown in Fig 2 (Table III) as insufficient numbers of stimulations were performed near the region of possible supernormality (1 8)

In one recording (case 19) (Fig 2C) the ACT ained constant until an increase occurred within msec of the AERP Lack of S_1–S intervals nger than AERP+34 msec made it impossible to judge if there was a supernormal conduction

Atrial atrioventricular conduction

The relation between the paced coupling intervals (S_1 S_2) and the response intervals (D_1–D) is depicted in Fig 3 for one individual with and another without signs of supernormal conduction The line of identity is shown in the figure At longer coupling intervals the two variables were near or on the line of identity As the premature coupling intervals approached the AERP the D_1–D_2 interval diverged more and more from the line of identity in other words when the prolongation of the ACT increased the response intervals got more and more distant from the initial S_1–S interval The same applied to the recordings with supernormal conduction but the divergence from the line of identity was less pronounced

The role of the atrium in the impulse propagation from the atrium to the ventricles was also investigated (Table III) In six recordings the stimulated impulses were conducted to the ventricles during the entire determination of AERP and no AVERP could be estimated As shown in Fig 3 however the D_1–D interval differed from the S_1–S_2 interval near the AERP Therefore the S_1–S_2 interval did not accurately portray the stimulation interval near the

Table IV *Delay in atrioventricular conduction in relation to the prolongation of impulse propagation within the atrium*

S=stimulus artefact D=start of depolarization of the right atrial MAP V=start of the QRS complex in the precordial ECG lead Basic=basic conduction time Max=maximal conduction time

Case no	AERP (msec)	S–D (msec) Basic	Max	S–V (msec) Basic	Max
11	274	45	116	200	370
19	366	20	26	150	180
21a	270	21	68	160	310
21b	248	20	76	190	300
33	314	52	60	190	350
40	218	52	74	210	400

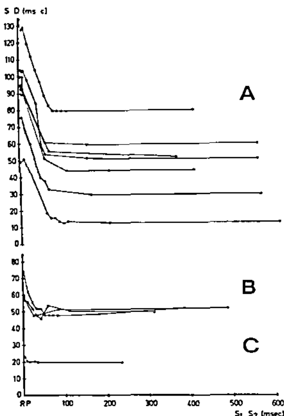


Fig 2 Atrial conduction time (S–D) in relation to paced coupling interval (S_1–S_2) in seven recordings with at least five determinations within 100 msec of the AERP and with at least one determination between 60 and 100 msec. A=recordings without signs of supernormal conduction. B=recordings with a supernormal phase of conduction. C=case. RP=individual refractory periods

detecting site. In these six cases the prolongation of the conduction time from the stimulus site to the ventricles was calculated (Table IV). The most pronounced delay occurred in or below the AV node. The percentage prolongation however was longer in the atrium than in the atrioventricular conducting system in four of these cases. In most of the other investigations the AVERP was considerably longer than the AERP and the prolongation of ACT was probably of minor importance in the evaluation of AVERP. In case 28 the AV conduction failed at a S_1–S_2 interval of 320 msec (AERP 310 msec) but the shortest D_1–D interval at the MAP electrode was 348 msec.

Errors of the method

The variation due to analysis of a single ACT was estimated from the results of double determinations in 15 recordings. The error of a single determination was calculated as

$$s=\sqrt{\Sigma \frac{d_i}{2n}}$$

(d_i=difference between two analyses, n=number of pairs). This variation was 1.05 msec giving a coefficient of variation of about 2%.

The intraindividual variation in ACT of the basic cycle length was calculated from 10 consecutive beats in 10 individuals. The standard deviation of ACT within an individual was 0.92 msec giving a coefficient of variation of about 2%. The recordings were accepted and considered to have a stable catheter position if all individual basic ACTs were within the 95% confidence limits.

DISCUSSION

In the present study ACTs have been measured from the onset of a pacemaker artifact to the onset of a MAP signal since the MAP recording accurately reflects the onset of the depolarization (12, 13). The term ACT thus includes both conduction properties and factors covered by the term latency (12), i.e. cellular characteristics round the stimulating electrode.

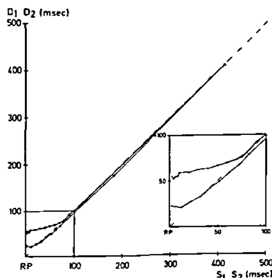


Fig 3 Relation between response intervals (D_1–D_2) and paced coupling intervals (S_1–S_2) in one recording with and one without signs of supernormal conduction. AERP+100 msec is magnified to the right. RP=individual refractory periods

Atrial conduction and heart rate

Several studies have shown that during atrial pacing with simultaneous His bundle recording (6 20) the P H interval successively increased at higher paced atrial rates but the H V interval remained constant In those studies the P H interval increased about 80 msec when the paced cycle length was changed from 800 to 400 msec and most of this increase was considered to be due to slowing of impulse propagation within the AV node In some of the investigations the role of the intraatrial conduction was not considered (20) Our investigation however indicates in addition a minor increase in ACT at higher paced heart rates A similar increase in intraatrial conduction time was observed in dogs (8) when pacing near the sinus node and recording near the AV node

Atrial conduction—atrial effective refractory periods

This study showed that there was a successive increase in ACT when the coupling interval between the basic and premature stimulus approached the AERP In the majority of cases the increase in ACT started within 80–100 msec of the AERP the greatest increase occurring in the last 50 msec This prolongation was of comparable magnitude for both long and short ACT of the basic cycle length The most probable explanation for this fact is that most of the increase in ACT took place near the stimulat pacemaker electrode In studies of human right muscle strips the conduction velocity was to be about 300 mm/sec for atrial muscle cells about 450 mm/sec for specialized conducting cells (9) Similar values have been found in the human fetal heart (10) The shortest basic ACTs in our study were 13–20 msec which would correspond to a distance of 1/2–1 cm Thus probably most of the ACT prolongation occurred within this distance In favour of this hypothesis is an investigation in humans (1) where pacing was performed close to the sinus node and recording near the AV node The maximal ACT increase in that investigation was about 50 msec which is comparable with our results One possible mechanism for this prolongation of ACT is that when the impulses are delivered near the refractory period the stimulated area is not fully repolarized and the resultant action potential will have a reduced depolarization rate and probably a reduction in propagation velocity of the impulse (13) Adjacent areas would thus be reached by the excitation later in the repolarization phase and finally during the phase of full recovery in which the conduction properties are optimal Local factors near the stimulating electrode included in the term latency (12) are also of importance Investigations concerning electrophysiological properties within the human right atrium have revealed a great variation in the duration of the MAP within the same atrium (2 3) These circumstances make it possible that parts of the prolongation in ACT might occur more distant from the stimulation site if the impulse reached cells close to their refractory periods Another mechanism responsible for the degree of prolongation of the impulse propagation might be the position of the two catheters with regard to the preferential conducting pathways of the atrium (16 17)

Supernormal conduction

The error in the determination of single and consecutive ACT is small Thus the method is sensitive enough to detect small differences in time We have regarded S_1–S_2 intervals below the 95% significance limit of the basic ACT as supernormal conduction This was found in three cases within AERP+20 to 200 msec It is possible that a mechanical origin such as catheter movements might be responsible for the changes in ACT but we think it is more likely that supernormal conduction is a true electrophysiological event within the atrium in some individuals

The prolongation of ACT close to the AERP was less pronounced in cases with supernormal conduction than in the other recordings In another nine investigations supernormal conduction may have existed but could not be verified as S_1–S_2 intervals where one could expect supernormal conduction were not investigated (1 8)

Some investigators have regarded the supernormal phase of conduction as a constant feature of specialized conducting cells within the atrium (4 5 8) others (17) have denied the existence of such cells With our technique it has not been possible to confirm or deny the existence of specialized conducting cells within the atrium The cells are considered to have a configuration of the action potential different from ordinary atrial muscle cells in that they have a prominant plateau (phase 2) and overshoot (9 14) A corresponding MAP configura-

tion was not found in our investigations On the other hand the MAP probably mirrors the electrical events in a number of cells (12 13) and some of these might have shown specialized conducting cell characteristics without affecting the general configuration of the MAP

Atrial atrioventricular conduction

When determining the AVERP the stimulus interval of importance is the one reaching the region of the AV node This interval however is not necessarily equal to the originally introduced S_1–S_2 interval near the sinus node Investigations in dogs (8) and man (1) have shown a slowing of atrial impulse propagation between the sinus node and the AV node as the S_1–S_2 intervals approached the AERP In both those studies the impulse detecting catheter was situated in the region of the AV node but the degree of ACT prolongation was similar to that in our study in which the impulse detecting MAP electrode was placed at different positions within the atrium but not close to the AV node It therefore seems likely that the increase in ACT noted at the MAP electrode is similar to that detected by electrodes near the AV node Neither the present technique nor the technique used in the study quoted (1) can reveal an increase in the velocity of impulse propagation These studies can therefore not yield any information about specialized conducting pathways within the atrium

In six determinations the AERP prevented determination of the AVERP i e there was still impulse propagation through the AV node within 2 msec of the AERP The D_1–D_2 intervals however were 6–71 msec above the originally introduced shortest S_1–S_2 interval Thus if the degree of conduction delay near the AV node is similar to that detected at the MAP recording site the increase in ACT made it impossible to obtain a D_1–D interval short enough to estimate the AVERP in spite of a possible shorter AERP i e the reduction in S_1–S produced a comparable increase in the D_1–D interval When determining the AVERP the response interval should therefore always be calculated and compared with the S_1–S interval (21) Calculation of response intervals however is sometimes difficult since the onset of atrial depolarization as recorded by conventional intracardiac electrodes is often difficult to determine especially when the premature A wave is in close relation to the previous QRS complex One advantage in using MAP recordings is that the onset of atrial depolarization can always be determined no matter how short the S_1–S_2 interval

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Ventricular Arrhythmias in Acute Myocardial Infarction

A Comparative Study on some Tests for Ventricular Arrhythmias

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ABSTRACT A continuous ECG recording has been made in 31 myocardial infarction patients during the first 24 hours after admission to hospital The number and severity of ventricular arrhythmias were recorded in great detail Before discharge from hospital the patients were submitted to 20 hours of ECG tape recording an exercise test on a bicycle ergometer and a static work test (handgrip) Another exercise test was performed one month after discharge During the first day in the Coronary Care Unit (CCU) all 31 patients had ventricular arrhythmias and in 27 of them the arrhythmia was classified as major (calling for treatment according to Lown s criteria) At the exercise tests 23 patients showed ventricular arrhythmias 12 of them considered as major No antiarrhythmic therapy was given during the investigation No correlation was found between the degree of arrhythmia during the first day in the CCU and during the exercise tests Tape recorded ECGs appeared to be inferior to dynamic exercise tests in the ability to disclose a latent tendency to ventricular arrhythmia Static work did not provoke any ventricular arrhythmias At a 2 year follow up 5 patients had died, 4 of them suddenly Examination of additional material on 11 patients with ventricular tachycardia or ventricular fibrillation during the CCU stay showed that 2 had died, but only one suddenly Frequency and severity of arrhythmias during the first day after the infarction seemed to correlate poorly to a persistent tendency to arrhythmias or to the risk of sudden death during the following 2 years A dynamic exercise test performed before discharge would appear to be more effective in selecting patients in need of long term prophylaxis However very few patients seem to need such a specific antiarrhythmic prophylaxis

Ventricular extrasystolic beats (VEBs) signify an increased mortality in the presence as well as in the absence of clinically recognizable heart disease (1 4) The increased mortality is especially pronounced in patients who have had a myocardial infarction and instead of being confined to the immediate postinfarction phase it lasts for several years (12 13) The mortality during the first year in patients surviving the acute phase of a myocardial infarction is said to be about 8% and half of these patients die suddenly probably due to ventricular fibrillation (VF) (12) Hence it would be of great clinical importance if patients with an increased tendency to ventricular arrhythmias could be identified at an early stage thus facilitating the institution of prophylaxis

It has been established that almost all patients with an acute myocardial infarction (AMI) exhibit ventricular arrhythmias in 70–80% of cases of such a degree that therapeutic intervention is considered necessary (11) The high incidence of ventricular arrhythmias during the early postinfarction period makes the decision with regard to long term prophylaxis difficult Nevertheless the severity of the initial arrhythmias has been used as a guide to the need of long term prophylaxis It must be stressed however that nobody has hitherto been able to show that the severity of the initial arrhythmias correlates with the occurrence of ventricular arrhythmias after the immediate postinfarction period On the contrary some investigators have found the long term prognosis for patients resuscitated from VF to be the same as that for patients with uncomplicated infarctions (7 16)

Lown and Wolff (9) demonstrated that routine ECGs and short ECG recordings for 1–3 min during rest gave insufficient information concerning the tendency to ventricular arrhythmias T

recorded long term ECG has been used as an alternative Ventricular ectopic activity can be identified much more often in this way Exercise tests are an even more effective way to demonstrate ectopic activity as shown by Kosowsky et al (5) They compared the incidence of VEBs during treadmill tests and during 10 hours of tape recording in patients with known ischaemic heart disease but without VEBs on routine rest ECGs During the tape recordings 27% of the patients had VEBs while 39% had VEBs during exercise

We have compared the frequency and severity of ventricular arrhythmias during the initial stage of AMI with the tendency to ventricular arrhythmias later on The arrhythmias during the first day in hospital were compared with arrhythmias during 20 hours of tape recording and during a standardized exercise test on a bicycle ergometer before dis charge The exercise test was repeated one month later Within the frame of this investigation we also evaluated the arrhythmogenic effect of static work using a handgrip test A 2 year follow up study has also been performed with investigation of causes of death

Table I *Clinical characteristics of the patie[nts] studied*

Sex (no of pats)	
Male	27
Female	4
Age (y)	
Mean	60
Range	42–70
Previous cardiovascular disease (no of pats)	
Angina pectoris	21
Myocardial infarction	4
Hypertension	15
Admission delay (h)	
Mean	6
Range	0 5–27
Severity of myocardial infarction	
Mean highest SGOT (U/l)	116
Infarction site (no of pats)	
Anterior	16
Posterior	4
Posterolateral	4
Indefinite	7
Heart volume (ml/m² BSA)	
Males >500 (no of pats)	6
Females >450 (no of pats)	1
Interval between admission and exercise test I (d)	
Mean	16
Range	10–23
Therapy during exercise tests I and II (no of pats)	
Digitalis	2
Diuretics	5
Digitalis and diuretics	2

MATERIAL

The study included all patients aged 70 or under who during a four month period in 1973 were discharged from the Division of Cardiology at the Department of Medicine Linkoping University Hospital having survived an AMI

Patients with persisting ventricular arrhythmias calling or long term antiarrhythmic therapy were excluded from the study as were patients with cardiac failure and those with complicating diseases which ruled out a bicycle ergometer test All patients were informed and their oral consent was obtained

METHODS

During the first days of hospital care the infarction pa tients were monitored in the Coronary Care Unit (CCU) by conventional means In addition the ECG was re corded on an 8-channel ink jet ECG recorder (Mingograph 81 Siemens Elema Stockholm) at a paper speed of 10 mm/sec The ECG signal was taken on line from the central monitoring unit of the CCU All continuous ECG recordings were analysed by one of us The analyses pertained to the time from admission to the start of antiarrhythmic therapy or at the most for 24 hours

Ventricular arrhythmias were defined as follows 1) VEB premature QRS complex with a duration of more than 0 10 sec a configuration differing from ordinary QRS complexes and without a preceding P wave Less than 5 and more than 5 VEBs/min were recorded separately 2) Paired VEBs (P) two successive VEBs 3) Multifocal VEBs (Mf) two or more VEBs with different QRS config urations 4) R on T wave type VEBs (R/T) a VEB starting within the first 85% of the QT time of the preceding QRS complex 5) Ventricular tachycardia (VT) three or more VEBs with a frequency of over 120/min 6) VF conven tional definition

Ventricular arrhythmias calling for treatment (referred to as major arrhythmias) were defined according to the criteria set by Lown et al (8) more than 5 VEBs/min Mf P R/T VT and VF In this article less than 5 VEBs/min is referred to as a minor arrhythmia

During the hospital period the patients received conven tional therapy including oxygen for the first 24 hours anticoagulant therapy pentazocine and/or hydro morphone atropine for analgesia diazepam for seda tion and in the event of heart failure digoxin and/or furosemide Lidocaine was used as the initial anti arrhythmic drug being substituted later on by procaine amide or phenytoin

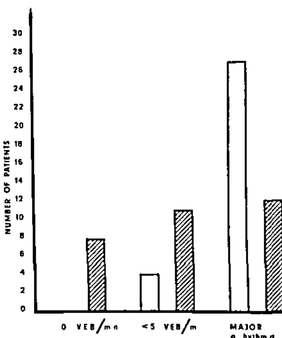


Fig. 1. Number of patients with and without ventricular arrhythmias during the first day in the CCU (□) and during exercise test I (▨).

The diagnosis of myocardial infarction required at least two of the following criteria: 1) Central chest pain lasting for more than 15 min. 2) Elevation of the appropriate enzymes. 3) ECG findings compatible with the diagnosis of myocardial infarction.

Patients were discharged from hospital after 2–3 weeks. The time spent in the CCU varied considerably, but in no case was it less than 24 hours.

During the last day in hospital an exercise test on a bicycle ergometer was performed (exercise test I). Antiarrhythmic therapy was discontinued at least 5 days before this test and relevant plasma determinations of the antiarrhythmic drugs previously used were made to ensure that no pharmacologically active concentration remained on the day of the exercise test. All the exercise tests were performed in the afternoon, at least two hours after the midday meal. Patients were instructed not to smoke during the two hours prior to the test. All patients were discharged on no antiarrhythmic therapy and a second exercise test was performed one month later (exercise test II).

During the exercise tests the ECG was monitored continuously with two bipolar chest leads. Before exercise the patients were in the supine position for 10 min and rested thereafter for 5 min on the bicycle. In exercise test I the initial work load was 10 W, increasing by 10 W every 30 sec to a maximum of 100 W, or until the heart rate increased to 110–120/min. In exercise test II the maximum work load was 150 W or until the heart rate increased to 130–140/min. After reaching the maximal work load, patients were requested to continue at this load for 5 min aiming at a steady state of work with a heart rate of 120 and 140/min in exercise tests I and II, respectively. The tests were ended with 5 min of supine rest, during which the ECG monitoring was continued. The tests were discontinued if angina pectoris, marked dyspnoea, fatigue or VT occurred, if there was a fall in the BP during the increased work load or if the diastolic BP exceeded 130 mmHg.

Before both exercise tests a static work test was performed using a handgrip. During this test ECG was recorded continuously and the BP was measured every minute. The patient's maximal grip was determined and he was then requested to compress the handgrip continuously for 3 min with one third of his maximal capacity.

A 20-hour ECG tape recording was made, either on the day before or after exercise test I, while the patients were pursuing normal activities in the ward. The tape analyses were performed by a trained technician and if any arrhythmias were found, they were checked by one of the authors.

Two years after the exercise tests all patients were followed up. The clinical information was obtained from hospital charts and also by family interviews, where appropriate.

RESULTS

During the chosen four month period of 1973, 34 patients (29 male and 5 female) fulfilled the set criteria. Three of them were excluded from the trial, one for orthopaedic reasons, one due to a persistent arrhythmia contraindicating withdrawal of antiarrhythmic therapy and one due to transfer to another department. Some relevant clinical and laboratory data on the patients are given in Table I. The serum digitalis level, estimated in all patients on digitalis therapy, was in no case within the toxic range.

First day in CCU ($n=31$)

No patient was completely free from ventricular arrhythmias.

Exercise test I ($n=31$)

The mean maximal work load was 70 W (20–100) with a mean maximal heart rate of 120/min (90–150). Twelve patients were unable to complete the test, 9 due to angina pectoris with or without dyspnoea, 2 due to dyspnoea and/or fatigue and 1 on account of VT. During the 10 min of supine rest and 5 min of rest on the bicycle, only 9 patients had arrhythmias, 8 less and 1 more than 5 VEBs/min. During exercise and during the 5 min rest after exercise, 12 patients exhibited major arrhythmias. Eight of the 15 patients who developed anginal pain had major arrhythmias.

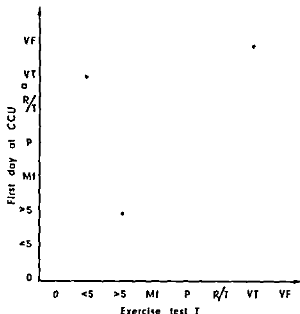


Fig 2 Number of patients with each type of ventricular arrhythmia during the first day in the CCU and during exercise test I

Fig 1 presents the incidence of arrhythmias during the first day in the CCU and during exercise test I A decline is seen in the number of patients with ventricular arrhythmias and also in the severity of the arrhythmias

Fig 2 shows the type of arrhythmia in each patient during the first day in the CCU and during exercise test I It is worth noting that of the 14 patients who initially had the most severe types of arrhythmias only four had major arrhythmias during exercise test I A decreased tendency to arrhythmias was observed in 21 patients and an increased tendency in five

Exercise test II (n=28)

Three of the 31 patients who performed exercise test I did not take part in exercise test II One was omitted due to cardiac failure one due to severe anaemia and in one patient antiarrhythmic therapy had been instituted during the intervening month The remaining 28 patients performed exercise test II with a mean maximum work load of 100 W (40–160) and a mean maximum heart rate of 134/min (100–155) Twenty-one patients discontinued the test 16 due to anginal pain with or without concomitant dyspnoea four due to dyspnoea and/or fatigue and one because of a pronounced increase in BP

During the 10 min of supine rest and 5 min of rest on the bicycle 22 of the 28 patients were completely free from arrhythmias Only one patient exhibited multifocal VEBs During exercise and during the 5 min of rest after exercise ten patients were free from VEBs while ten had major arrhythmias

During exercise test II 18 patients developed angina pectoris seven of whom had major arrhythmias The severity of arrhythmias occurring in patients with anginal pain in both exercise tests did not differ from that in patients without angina

Fig 3 shows the types of arrhythmia during exercise tests I and II There is a rather good correlation both for the number of patients with arrhythmias and for the severity of the arrhythmia The arrhythmia increased from minor to major in only three patients

If exercise tests I and II are broken down into one minute intervals one can compare them in terms of the number of intervals free from VEBs expressed as a percentage of the total number of intervals Comparing the two tests in this way gave a maximum difference of 15% This difference was found during the rest period after exercise during the other parts of the exercise tests the difference was much smaller amounting to only 1–5%

No complications occurred during the exercise tests In no case did the anginal pain require treatment All patients were free from symptoms within 10 min

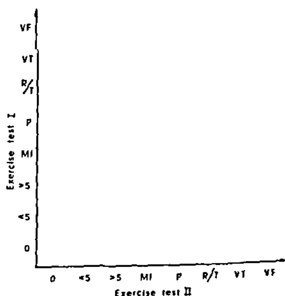


Fig 3 Number of patients with each type of ventricular arrhythmia during exercise tests I and II

Table II *Frequency of each type of arrhythmia during the various investigative procedures*
Only the most severe arrhythmia at each procedure is presented

	Type of ventricular arrhythmia							
	No arrhythmias	<5 VEBs/min	>5 VEBs/min	Mf VEBs	P VEBs	R/T VEBs	VT	VF
First day at CCU (n=31)	0	4	4	8	6	2	6	1
Exercise test I (n=31)	8	11	2	5	4	0	1	0
Exercise test II (n=28)	9	8	2	5	4	0	0	0
Tape recorded ECG (n=24)	9	10	2	2	1	0	0	0
Static work (n=21)	0	0	0	0	0	0	0	0

Tape recorded ECG

Altogether 27 patients were studied Due to a technical error only 24 recordings could be analysed giving a total of 480 hours of tape recording Another 68 hours of tape recording were unfortunately damaged due to electrical noise during analysis The total number of tape recorded ECGs studied therefore amounted to 412 hours

Ten patients showed no arrhythmias four of them had minor and one had major arrhythmias during exercise test I Nine patients had occasional VEBs during tape recording four of them had major arrhythmias during exercise test I Major arrhythmias were found in five patients during tape recording and at exercise test I four of these had the same type of arrhythmia One patient had only occasional VEBs

Static work (handgrip test)

Twenty-one patients performed the handgrip test in conjunction with exercise tests I and II All patients found the test strenuous but none developed dyspnoea or angina pectoris The tests caused a considerable increase in both systolic and diastolic BP the mean systolic increase amounting to 35 mmHg (0–70) during test I and 39 mmHg (0–80) during test II The corresponding diastolic increase was 21 (0–45) and 25 (5–50) mmHg respectively Diastolic values of 150–155 mmHg were noted in several patients the two most pronounced increases being from 160/110 to 230/155 and from 135/75 to 215/120 No corresponding increases were seen during the exercise tests No arrhythmias were seen During the first minute after the handgrip test two patients showed occasional VEBs

Table II lists the most severe arrhythmias found during the various investigations The high frequency of arrhythmias during the first day in the CCU and the similarity of incidence of arrhythmias between exercise tests I and II are also evident Only one patient developed one attack of the three most severe types of arrhythmia VT during any of the tests

Modes of death

At 2 year follow up 5 of the 31 patients had died 4 suddenly (Sudden death is defined as death occurring within 24 hours of the onset of acute signs or symptoms) Table III shows the type of ventricular arrhythmia demonstrated by these patients at various stages of the investigation None had demonstrated serious arrhythmias at any stage All of these patients had prodromal central chest pain before death and reinfarction was verified in 3 patients who died after admission Many of the 31 patients were treated with β blocking agents because of angina or hypertension but none had any other specific antiarrhythmic therapy

DISCUSSION

All antiarrhythmic drugs available today have side effects of such severity that they cannot be

Table III *Type of ventricular arrhythmia during the various investigative procedures in patients who died suddenly*

Pat no	First day at CCU	Exercise test I	Exercise test II	Tape recorded ECG
1	<5	>5 Mf	Mf	<5
2	>5 Mf P	0	<5	0
3	Mf	0	Mf P	<5
4	<5	P	<5	<5

ignored especially when they are compared with the efficacy of the drugs (6) This is especially true during long term therapy The decision as to long term prophylaxis against ventricular arrhythmias is thus serious and should be made on a more secure basis than at present As stated the occurrence of ventricular arrhythmias during the first days after a myocardial infarction probably does not constitute such a foundation An exercise test at the time of discharge from hospital would seem to be more reliable

The high incidence of ventricular arrhythmias *during continuous ECG recording on the first day* after an AMI is well known and was also found in our study

If conventional oscilloscope surveillance is used alone the number of detected arrhythmias decreases considerably, generally to below 50% of those detected by continuous ECG recording (14)

The exercise tests showed that during the 15 min of rest before the test a very low number of VEBs occurred and no major arrhythmia was recorded During exercise and during the first few minutes *afterwards there was an increase both in the fre*quency and in the severity of arrhythmias There was no correlation between the frequency and severity of ventricular arrhythmias during the first day in the CCU and during exercise test I On the other hand a good correlation was demonstrated between the two exercise tests During exercise test II the frequency of VEBs was higher but the degree of severity was not The increase in incidence may have been due to the higher work load as indicated by the fact that only a few patients completed this test

Since ventricular arrhythmias occur so frequently during the acute stage of an infarction one would assume a high frequency of arrhythmias in patients *developing angina pectoris during exercise tests* compared with those without anginal pain However we did not find anything to support this assumption This is in line with the findings of Crawford et al (2)

The tape recordings in 24 of the patients demonstrate that this procedure is of less value than an exercise test in detecting ventricular arrhythmias in accordance with the findings of Kosowsky et al (5) In contrast Crawford et al (2) found continuous monitoring more effective than exercise testing *in detecting* significant arrhythmias in 60 patients with previous myocardial infarction In their study 30% of patients had frequent VEBs during a submaximal treadmill exercise while 37% had significant arrhythmias during 10–12 hours of continuous ECG monitoring Our study was undertaken close to the actual myocardial infarction and the patients were tape recorded under hospital conditions In the study of Crawford et al, however in general more than a year had passed since the infarction *and the patients were monitored during routine* daily activity out of hospital This may explain some of the discrepancy and it is possible that a comparison between exercise test II and tape recording on an outpatient basis in our material would have shown a smaller difference Tape recording is a simple procedure without inconvenience to the patient The major problem arises in the analysis of the tapes A well trained technician is needed to obtain reliable results and even then VEBs of the R/T type or multifocal VEBs are hard to find especially if the frequency of VEBs during the entire tape recording period is high making a continuous print-out practically impossible Certainly these shortcomings will be surmounted when reliable *means have been developed for automatic rhythm* analysis Today telemetry seems to be a more convenient screening method during the stay in hospital even though it does not permit 100% detection of arrhythmias

Static work has been shown by several investigators to be a simple method of testing left ventricular function (3 10 15) It has been shown by invasive techniques that the intracardiac pressure changes during static work correspond to those during moderate dynamic work Some investigators have reported arrhythmias to occur during static work (10)

We have not found any investigations in which the effect of static work has been studied in patients *in the immediate postinfarction period* In spite of a very severe increase in systolic and diastolic BP during static work, none of our patients showed ventricular arrhythmias nor did any patient de*velop angina or dyspnoea during this test*

It is interesting to note that of the 9 patients with the most severe arrhythmias during the initial stay i e VEBs of the R/T type VT and VF only 2 patients in exercise test I and *1 patient in exercise* test II had major arrhythmias The 2 year follow-up also showed that none of these 9 patients had died suddenly However of the remaining patients without severe arrhythmias demonstrated in the CCU 4

had died Although a small sample the figures support the hypothesis that the initial type of arrhythmia is of little use as a prognostic index or as a basis for determining the need for long term specific prophylaxis against ventricular arrhythmias This opinion is supported by investigations completed after the initial study We continued using the exercise test at the time of discharge in 11 patients showing VT or VF during the acute stage of the myocardial infarction Three patients in whom VF had occurred initially were completely free from arrhythmias during the exercise test Of 8 patients with VT only 2 developed paired or multifocal VEBs during the exercise test Six patients had minor arrhythmias or were completely free from arrhythmia At the 2 year follow up only 2 out of these 11 patients had died one suddenly

We consider that a standardized exercise test performed before discharge of myocardial patients from hospital may be a more effective way of discriminating clinically important and persisting arrhythmias after a myocardial infarction None of our patients showed any more serious arrhythmias at the exercise test nor did they seem to die from arrhythmia All the 4 patients who died suddenly had complained of central chest pain of at least one hour s duration prior to death Furthermore in 3 of these patients a reinfarction was verified and they died in cardiac failure after admission to hospital

Some patients were treated with β blocking agents but as they received these drugs because of angina pectoris or hypertension and not because of arrhythmia this may not influence the conclusions drawn above Most of the patients despite their sudden death had prodromal central chest pain of at least one hour s duration indicating reinfarction However it is conceivable that reinfarction may be initiated by ventricular arrhythmia Therefore further studies are needed in order to investigate the value of specific long term antiarrhythmic therapy in preventing out hospital sudden death and reinfarction

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Regional Coffee Consumption and Mortality from Ischemic Heart Disease in Finland

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ABSTRACT In Finland the mortality from ischemic heart disease is highest in North Karelia and lowest in Southwest Finland In this study the consumption of coffee has been measured from the total sales in the different areas of Finland to see if it correlates with the mortality from heart disease North Karelia had higher coffee sales per inhabitant than Southwest Finland This finding reinforces the view that carefully controlled dietary studies are needed to establish the role of coffee in the mortality from ischemic heart disease

Finland has the highest mortality from ischemic heart disease among working age males (23 24) But within Finland there are great regional differences (10 16) mortality is highest in the province of North Karelia in Eastern Finland (with an age standardized index of 145 if the national mortality is 100) and lowest in the province of Turku Pori in Southwest Finland (index 82) (16)

The consumption of coffee has recently been added to the various factors which are implicated in *ischemic heart disease (3 7) This has been debated* extensively and critics have referred to studies in which no correlation was found between ischemic heart disease and coffee drinking (4 9 11 13)

Several risk factors including higher serum cholesterol concentrations smoking and higher fat and sugar consumption have been reported in the North Karelian population in comparison with Southwest Finland (8 10 15) But there are no reliable data on the consumption of coffee in these two areas

The purpose of this study was to find out whether the consumption of coffee in North Karelia differs from that in other parts of the country especially Southwest Finland

METHODS

Total sales There are nine coffee companies in Finland but the five biggest have 99% of the market They are included here as companies A B C D and E which in 1971 had 31 20 13 22 and 13% respectively of total sales Regional sales figures for coffee were obtained from four of these companies for the period 1969–73 The years 1970 1971 and 1972 were chosen for analysis The sales of the biggest coffee company (A) in 1972 also included the sales for Jan 1973 Company C's complete regional wholesale distribution was not available for 1972 Company E s regional distribution of sales was obtainable only for 1974 The regional sales in 1970–72 were estimated from the distribution of sales in 1974

The sales regions were defined differently by each company For the regions of each company the following indices were counted annual sales of coffee per inhabitant and annual sales of coffee per inhabitant over 15 years old The population of each region was obtained by adding up the populations of the individual communes (18 19) To calculate total coffee sales the indices for the different companies were summed for each area The results were based on the regional divisions used by company A (Fig 1) The regions of company B were rather similar Company C's sales were counted from the sales of individual cooperative shops to cover the areas in Fig 1 Companies D and E used larger sales regions than those in Fig 1 If more than one area given in Fig 1 corresponded with a region of companies D or E the same index was used for these areas If an area in Fig 1 was between two regions of companies D or E the mean of the indices of these regions was used Thus the sales of companies D and E dilute the differences between the areas

Retail sales A private market research organization Marketindex continuously collects data on sales from retail shops using inventories from a representative sample of shops (12) In 1972 the retail sales represented 74% of the total sales the rest of the coffee was sold wholesale in bulk to caterers such as restaurants work places and hospitals The sales regions used by Marketindex differ somewhat from those given in Fig 1 The region corresponding to Southwest Finland was only the southern most half of area 1 and the region corresponding to North Karelia included in addition to area 6 the southern half of area 7

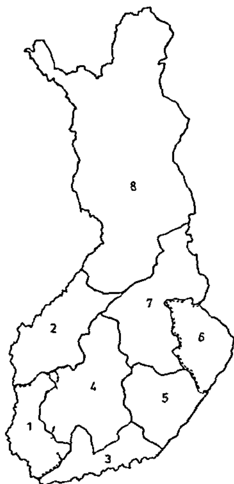


Fig 1 The areas used for counting coffee consumption =boundaries of the provinces Turku Pori (*1*) and North Karelia (*6*)

Households purchases Finland s Gallup continuously surveys domestic purchasing habits using a representative sample of households (21) According to this source the domestic consumption of coffee corresponded to 73 % of the total sales in 1972 Regions used by Gallup were rather unsuitable for the present study since areas 6 7 and 8 in Fig 1 formed a region The region corresponding to Southwest Finland covered the southernmost half of area 1 and the western part of area 4

RESULTS

Table I lists the sales areas of the Finnish coffee companies according to the amount of coffee sold in the three years Most coffee is used in North Karelia (area 6) and least in Southwest Finland (area 1) if coffee sales are counted per inhabitant The differences between the areas amount to 1 3–1 7 kg from 1970 to 1972 When coffee sales are counted per inhabitant over 15 years old coffee sales in North Karelia are not the highest but notably higher than those in Southwest Finland The differences in sales range from 1 5 to 2 1 kg in 1970–72

Table I *Ranking of the sales areas of Finnish coffee companies according to the amount of coffee sold per inhabitant and per inhabitant over 15 years old in 1970–1972*

The numbers of the areas refer to Fig 1 the amounts of coffee sold (kg) are given within parentheses

1970	1971	1972*
Per inhabitant		
5 (10 6)	6 (10 2)	6 (11 3)
6 (10 3)	5 (10 1)	8 (10 9)
8 (10 2)	8 (9 7)	5 2 3 (10 1)
2 (9 6)	2 (9 4)	4 (9 8)
4 (9.5)	7 (9 2)	7 *1* (9 6)
7 (9 3)	3 (9 0)	
3 (9 1)	4 (8 9)	
1 (9 0)	*1* (8 6)	
Per inhabitant >15 years		
8 (14 2)	8 (13 6)	8 (15 2)
5 (13 8)	5 (13 3)	6 (14 4)
6 (13 1)	6 (12 9)	2 (13.5)
2 (12 8)	2 (12 5)	5 (13 1)
7 4 (12 4)	7 (12 3)	3 (13 0)
3 (11 7)	4 3 (11 6)	7 4 (12 8)
1 (11 6)	*1* (11 1)	*1* (12.3)

Part of the wholesale of company C is lacking

Since the sales figures of company E were not as accurate as those of the other companies the results were also considered without company E This method of calculation gave practically the same order of sales volume between the areas

The correlation coefficients were computed between the amounts of coffee sold in an area and the age standardized mortality from ischemic heart disease (16) in the province with the best fit When the coffee sold was counted per inhabitant the correlation coefficient (*r*) was 0 655 per inhabitant over 15 years old *r* was 0 526

The differences in retail sales between the areas corresponding to North Karelia and Southwest Finland were not remarkable When counted per inhabitant just as much coffee (9 kg) was sold in the two areas As for the retail sales per inhabitant over 15 years old North Karelia scored 10 5 kg coffee and Southwest Finland 10 1 kg The highest sales were in Northern Finland and the lowest in Southern Finland

Domestic purchases were highest in Northern Finland (7 9 kg per inhabitant and 10 6 kg per in

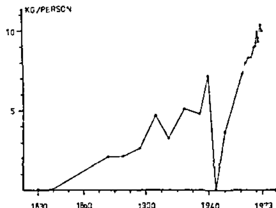


Fig 2 Sales of coffee per inhabitant in Finland in 1830–1973 Sources references 5 6 19 22

habitant over 15 years old) In the area of South west Finland the corresponding figures were 7 7 and 9 8 The sales of this area were the second highest of the five sales areas of Finland s Gallup

DISCUSSION

Coffee was introduced into Finland in the middle of the 18th century (6) In the 19th century its con sumption increased rapidly (Fig 2) In the 1960s the apparent consumption (imports) of coffee in Fin land was among the highest in the world second only to Sweden and Denmark (17)

We found regional differences in total coffee sales in Finland The two areas in Finland with the high est and lowest mortality from ischemic heart dis ease North Karelia and Southwest Finland also had the highest respectively lowest total sales of coffee per inhabitant Also a positive correlation was found between mortality and coffee sales but this is only suggestive since the provincial bound aries only roughly correspond to many of the areas of coffee sales

Because children use less coffee than adults and because the relative proportion of children varies in different regions of Finland coffee sales were also calculated per inhabitant over 15 years old In such statistics coffee consumption appeared to be high est in North Finland (with a large child population) but the differences between North Karelia and Southwest Finland remained equally large

The regional differences in the retail sales of cof fee or in its domestic consumption were not consist ent with the differences in total sales The con sumption directly through wholesalers mainly de scribes the amount of coffee consumed outside homes and apparently this varies in different parts of Finland Thus the data available from Mar ketindex (retail sales) or Finland s Gallup (domestic consumption) are not valid for measuring the amount of coffee consumption

Earlier studies on the correlation between re gional coffee consumption and mortality from ischemic heart disease have been contradictory According to two separate interview studies it seems that men in North Karelia drink more coffee than men in Southwest Finland (6 0 versus 5 6–5 9 cups/day/person) but women drink less (5 3 versus 5 9–6 0) (20) But as the studies were conducted separately and with different methods comparisons cannot be reliable Armstrong et al (1) have given correlation coefficients between mortality from ischemic heart disease and coffee consumption in nine regions of England Wales and Scotland in 1969 No positive correlation was found

The correlations between different countries are even more unreliable than comparisons within a country (see e g 1) yet they are of some interest Armstrong et al (1) found positive correlation be tween male ischemic heart disease mortality in 1969 and coffee consumption in 1955–57 but not be tween mortality in 1969 and coffee consumption in 1965 Brummer (2) found no significant correlation between coronary mortality and coffee consump tion Palotas (14) reported a high correlation be tween coffee consumption and deaths from ar teriosclerotic and degenerative heart disease in 1965–66

Our finding that more coffee was sold in the area with high mortality rate from ischemic heart dis ease need not mean that a similar correlation exists at the level of individuals It is possible that in North Karelia all persons or particularly those who do not have ischemic heart disease drink much coffee Besides this inherent drawback to ecologic correlations this study has some other meth odologic weaknesses which should be consid ered Firstly the sales regions of two coffee com panies were very large and their sales thus dilute and possibly distort the results Yet distortion is not probable because the exclusion of the company with the largest and least reliable figures did not noticeably affect the results The second possible limitation is that it is not known whether all coffee sold was used in the area concerned For example the high coffee sales in Northern Finland may be

partly due to border trade with Sweden and Norway

The present work surveys coffee consumption and mortality from ischemic heart disease cross sectionally This is relevant if coffee has immediate effects, such as changes in blood coagulation or cardiac arrhythmias on the ischemic heart disease *If the effects are gradual or cumulative such as an* increase in coronary sclerosis the cross sectional approach might not be valid because coffee consumption may have varied over time Such a variation is certainly possible—even within the three years surveyed in this study, coffee consumption was not constant However as the sales areas of different companies were not equal this variation may also be due to the changes in the market for the different companies or to other inaccuracies in counting the results

In spite of these drawbacks the sales figures do contribute greatly to the knowledge of coffee consumption and are more reliable than the data obtainable from interviews which are affected by poor memories and untruthful reporting In addition the measure used in previous studies the cup of coffee is very inaccurate the volume of a cup and the strength of coffee may vary

As Armstrong et al (1) have noted Associations seen between commodity consumption and ischemic heart disease need not reflect causal relationships but serve principally as pointers to further research Associations suggested by these techniques need to be tested by carefully controlled dietary studies

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Aetiology of Febrile Mucocutaneous Syndromes with Special Reference to the Provocative Role of Infections and Drugs

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ABSTRACT The survey covers 266 patients with febrile mucocutaneous syndromes A brief account of the highly varied clinical picture is given The syndromes constitute an allergic reaction which in most drug provoked cases appeared as a late immunological reaction of serum disease type for the rest sensitization had probably often taken place An allergic disposition could be traced in 34 % of the cases Infections and drugs were the causative factor in 235 cases (88 3 %) Infections accounted for 25 2 %, drugs for 36 1 % and both together for 27 1 % An account is given of the provocative bacterial and viral infections and of the role of drugs in cases with and without infection Among the remaining 31 cases an infection and/or drug may have been the provocative agent in 16 (6 %) whereas 15 (5 6 %) may have been due to alimentary and other factors

It has long been known that mucous membranes of the orifices of the body may also be involved in for example erythema multiforme exudativum But it was some time before this circumstance attracted any particular interest In 1916 Rendu (14) described a severe condition with attack of all orifices which was later called by Fiessinger et al (5) ectodermose erosive pluriorificielle But not until the report by Stevens and Johnson (17) in 1922 did the disease become the subject of greater interest These authors considered it to be a specific infection of unknown nature

Numerous publications on the subject have since seen the light of day and are met with under widely differing titles (20) The nomenclature has been confusing but most recent work is found under the name Stevens Johnson syndrome The justification for this is questionable since the syndrome had been described earlier and furthermore is considerably more varied than the original description indicates Most authors state the aetiology to be unknown But many hold drugs to be responsible while the role of infections has fallen into the background

I have decided in favour of a name which characterizes the clinical symptoms—febrile mucocutaneous syndrome The diagnosis requires fever exanthema and affection of at least two of the orifices In my first publication (18) in 1948 covering six cases no drug could be held responsible in two The investigations have been undertaken over more than a quarter of a century (1945–71) and the results are presented here

MATERIAL AND METHODS

Material This consists of 266 patients 128 male and 138 female aged 10 months–81 years (56 % 0–19 years 21 % 20–39 years 12 % 40–59 years 11 % 60 years and above) The patients had been admitted to the Roslagstull Hospital for Infectious Diseases and all have been examined by me

The *clinical picture* was characterized by an often high and lengthy fever of about 10 days duration with small daily variations and slow decline The highest temperature was 42 C above 40°C in 43 % of the cases The syndrome exhibited 17 clinical variants with involvement of all orifices in 11 % (ectodermosis erosiva pluriorificialis) of four in 12 % three in 34 % and two in 43 % (36 % in the form of Stevens Johnson syndrome—skin mouth eyes)

In 50 % of the cases the exanthema was of maculopapulous type usually neatly symmetrical often haemorrhagic Erythema multiforma exudativum was present in 33 % morbilliform rash in 8 rubelliform in 6 and ordinary erythema in 3 % Of the oral lesions (98 % of the cases) 38 % were of maculopapulous stomatitis type 20 % pseudomembranous 13 % aphtous 9 % vesiculous and 20 % glossitis plus gingivitis Typical crustous black lips

were recorded in 20% of the patients Of the eye symptoms (93% of the cases) 59% consisted of catarrhal conjunctivitis 37% purulent and 4% pseudomembranous Keratitis developed in 3 2% subconjunctival haemorrhage in 2 8% of the patients The nose symptoms (34% of the cases) consisted of scabby sores the genital symptoms (40%) of balanitis or vulvitis in 61% erosions 39% and the anal (25%) of swelling and rubor in 35% erosions 65%

The clinical picture in other respects bore the stamp of the underlying infection when present Despite the often severe and lengthy illness the prognosis was largely favourable Only two patients died one cardiac patient and one with secondary infection

Investigations Extensive investigations have been necessary both for detection and exclusion of acute infection Methods for detection of viral infections did not become available until the 1950 s Cultures have been made in 130 cases (113 from faeces 49 from cerebrospinal fluid 55 pharyngeal washings 12 skin blisters) and serological tests in 151 particularly complement fixation against a number of viruses (herpes simplex adeno polio tick borne encephalitis influenza parainfluenza respiratory syncytial virus measles mumps) as needed in the individual case At least a fourfold rise of titre has been required for a positive result A positive result both in culture and serologically occurred only in cases of infection with herpes simplex and adenovirus Heterophil agglutination of sheep blood cells has been examined in 186 cases and was positive in all cases of mononucleosis

As regards bacterial infections cold agglutination was done in 227 cases all 23 Mycoplasma cases were positive and in 11 of them complement fixation was done as well With few exceptions antistrepto and antistaphylolysins were determined and in more than half of the patients antipneumolysin A more than doubled titre was required for a diagnosis of acute infection—in several cases a dye test against toxoplasmosis was also made

RESULTS

Earlier investigations

The assessment of the entire material is based on the results of earlier studies In 1957 a report was published on systematic attempts to treat scarlatina and other streptococcal diseases with the then new probenecid penicillin drugs (19) This combined drug proved extremely inappropriate multiplying the side effects which might have been caused by each constituent Thus exanthema occurred in a large percentage of cases usually in combination with fever and in many cases with mucous symptoms even up to the appearance of febrile mucocutaneous syndrome This could accordingly be interpreted as a marked manifestation of an allergic reaction on an immunological basis in analogy with the serum disease reaction

The syndrome did not however always occur as a late allergic reaction of this kind The question of the role of drugs was considered in a later study (20) It seemed that the thrombocyte test might be of value A dose of a suspected drug would in the event of a positive result reduce the number of thrombocytes by at least 20% The trials showed that still greater clarity could be gained from the occurrence of clinical reactions in the form of mild exanthema sometimes also conjunctivitis and fever

Provocation tests were made in 29 cases with positive results in 19 (thrombocyte test in 3 clinical reaction in 10 and both in 6) The following provocative drugs were found sulpha 6 salazopyrine 1 probenecid penicillin 5 penicillin V 1 sulpha + penicillin 1 acetyl salicylic acid 2 codeine 1 Saridon® 1 and quinidine 1 In five of these positive cases only a few doses of the drug had been given which suggests a certain sensitization In some cases one ingredient of a drug for example codeine induced a reaction in others—such as Saridon—only the composite drug In 8 of the 10 negative cases the following infections were found Mycoplasma 4 serous meningitis 2 mononucleosis 1 streptococcal tonsillitis 1 In the light of the remarks below this suggests that the infection and not the drug was the provocative agent in these cases

The total material

As an allergic element is assumed to play a pathogenic role it is of interest to recognize that in 90 of the 266 patients (34%) there were anamnestic data indicative of an allergic disposition (hay fever and/or asthma 22 eczema 20 urticaria 7 alimentary hypersensitivity 24 drug reactions 17) In more than 75% of the cases provoked by drugs the type of reaction can be assigned to the late allergic immunologically originating form if one takes the liberty of setting the limit for the occurrence of such an antigen–antibody reaction at more than 3 days of continuous therapy When the drug has been given for a shorter time there is a greater probability that sensitization has taken place with an accelerated effect in consequence

In order to be able to evaluate the respective significance of infection and drug in the total material lists have been drawn up primarily of all patients with verified untreated infections (Table I) and of treated patients without infection (Table II)

Table I shows that the syndrome appeared in

Table I *Syndromes in cases with verified infections*

Infection	No of cases	
	Untreated	Total
Haemolytic streptococci	12	50
Pneumococci	0	6
Salmonella typhimurium	3	3
Paratyphoid B	0	1
Urinary tract infections	0	14
Mycoplasma pneumoniae	7	23
Mononucleosis infectiosa	4	29
Adenovirus 7	3	7
Herpes simplex	3	7
Vaccinia	3	3
Hepatitis acuta B	1	1
Meningitis serosa	4	11
Total	40	155

Table II *Syndromes caused by drugs*

Drugs	No of cases
Butazolidine	6
Oxyphenbutazone	8
Phenytoin	6
Mephenetoin	3
Sulpha	4
Salazopyrine	3
Barbiturates	6
Acetyl salicylic acid	3
Mercury	2
Ampicillin codeine meprobamate Doriden® Revonal® Vilexin+Salures (one case each)	6
Total	47

cases both of bacterial and viral infection The streptococcal cases were distributed as follows scarlatina 6 tonsillitis 42 erysipelas 1 impetigo 1 Pneumococci caused meningitis in 1 case pneumonia in 1 and infections of the upper respiratory tract in 4 Of the urinary tract infections 2 cases were classified as cystopyelitis 8 cystitis and 4 bacteriuria Infections caused by Mycoplasma pneumoniae gave rise to 16 cases of pneumonia 7 of upper respiratory infection All cases of mononucleosis had a positive heterophil agglutination and may thus be considered to have been caused by Epstein Barr (EB) virus The case of hepatitis was of B type

It is also evident that in 40 cases no drug had been given before the appearance of the syndrome these were cases of infection caused by haemolytic streptococci Salmonella typhimurium Mycoplasma pneumoniae EB virus adenovirus 7 herpes simplex vaccinia and hepatitis B Probably too a viral infection was responsible for the cases of serous meningitis even if this could not be proved (cultures and serological tests in 9 of the 11 cases yielded a negative result) No case of pneumococcal or urinary tract infection on the other hand was untreated and the drugs used were of a type which alone can provoke the syndrome These bacteria appear to lack allergenic potency

Syndromes caused by drugs in cases where an infection was ruled out are listed in Table II Butazolidine and its derivatives are in the lead followed by antiepileptics As regards sulpha it may seem remarkable that it was given in the absence of infection One patient took 50 tablets of Elkosin® in the belief that they were vitamins two others took sulpha prophylactically against meningococci and the fourth patient had collagenosis Salazopyrine had been given to patients with ulcerous colitis arthralgia and acne Barbiturates and acetyl salicylic acid also occupy a fairly prominent position in this context Of the two mercury cases one patient received local treatment with mercurial ointment and the other used oral tablets containing $HgCl_2$ (blistering in skin test) One patient had used the ampicillin tablets for protection against influenza Thus both sulpha and penicillin can cause the syndrome in the absence of infection

Table III shows the drugs used in all 156 cases of treated infections 115 with verified diagnosis Penicillins occupy the foremost place These were of all types though ampicillin and the combination with probenecid were the most common The combi-

Table III *Drugs used in infections with syndrome*

Drugs	No of cases
Penicillin	58
Sulpha	44
Penicillin+sulpha	18
Penicillin/sulpha+acetyl salicylic acid	6
Sulpha+butazolidine	2
Penicillin/sulpha+tetracycline	2
Tetracycline	1
Acetyl salicylic acid	18
Barbiturate	[illegible]
Meprobamate Doriden® Sandon® quinine (one case each)	[illegible]
Total	[illegible]

nation with sulpha is equally unsuitable Sulpha comes a good second The long acting types had been used in 46% of the cases It is striking that only in one case could an antibiotic of broad spectrum type—tetracycline—probably be held responsible Otherwise as is seen some of the drugs are represented which by themselves can cause the syndrome

But one cannot conclude from these tabulated data that the drug has always been a decisive or contributory cause of the syndrome On the basis of the preceding remarks concerning the significance of certain infections the nature of the drug and the length of treatment and of the results of the provocation tests however it can be established with reasonable certainty in most cases whether the drug the infection or both to the same degree may have been the decisive factor

The result of this analysis is shown in Fig 1 Infections have been the certain cause in 15% of cases the probable cause in 10 2% altogether in 25 2% The corresponding figures for drugs were 17 7 and 18 4% total 36 1% Both may have been equally responsible in 27 1% In 16 cases (6%) it is uncertain whether infection and/or drug played any role In 15 cases (5 6%) both infection and drug could be ruled out Alimentary and other factors may have been operative (in one case crab in two elderberries and in two cases detergents)

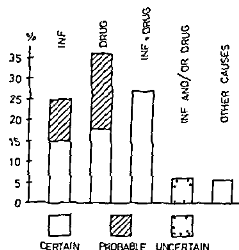


Fig 1 Aetiology of febrile mucocutaneous syndromes

DISCUSSION

It is quite natural that in considering the aetiology of febrile mucocutaneous syndromes interest has centred on the role of drugs in view of the possibility of taking prophylactic measures and of eliminating certain drugs and combinations of drugs It is nevertheless important to be aware of the significance of infections in this context and not to discriminate drugs unjustifiably The question also has a legal aspect

The provocative role of drugs is often manifest This has been demonstrated in numerous publications and in a major survey by Bianchine et al (1) In the present cases without infections butazolidines antiepileptics sulpha salazopyrine barbiturates, acetyl salicylic acid mercury etc have been among the causative factors In cases with infections the sulpha compounds especially the long acting have attracted the greatest attention especially after a publication by Carroll et al (3) which resulted in the issue of a warning to physicians in the US In reaction thereto Bianchine et al published their survey in which they denied this special risk and instead emphasized the role of the penicillins In a retrospective study of drug side effects reported to the Swedish National Board of Health and Welfare Bottiger et al (2) demonstrated that a considerable proportion of these side effects were caused by long acting sulphonamides The risks are probably greater with these agents owing to their slow excretion they accounted for 46% of the syndromes among sulpha treated cases in the present material The penicillins nevertheless occupied first place Combined drugs of the penicillin–sulpha and penicillin–probenecid type manifestly constituted a special risk Otherwise quite naturally patients with infections had received many of the drugs which can cause the syndrome in the absence of infection

Many authors have suspected that infections may be responsible for the appearance of the syndrome Coursin (4) demonstrated the probability of a relationship with streptococcal infection In the present material there are no less than 50 cases of streptococcal infections As regards Mycoplasma pneumoniae Ludlam et al (9) reported a high titre in complement fixation in five cases In connection therewith I reported 16 cases with infection positive to cold agglutination (21) to which may now be added seven additional cases (altogether 11 with positive complement fixation) Mycoplasma pneumoniae has since been isolated in one case each by Foy et al (7) Sieber et al (16) and Sanders et al (15) Apart from these two bacterial infec-

tions the syndrome has occurred in three patients with salmonellosis typhimurium

As regards viral infections Grasso and Petrone (8) reported a rise of titre of complement fixing anti bodies against adenovirus in two patients I have earlier published 6 cases (22) to which one more has now been added Adenovirus 7 was found in five and all cases were verified serologically The significance of herpes simplex infections as cause of erythema multiforme exudativum has often been discussed and has been thoroughly illustrated by Nasemann (12) It may then be natural to conceive of a relation also with mucocutaneous syndrome Tolentino and Semach (25) Foerster and Scott (6) and Pandi (13) have described one such case each The difficulty associated with herpes infection lies in the fact that the virus causes specific local lesions at the body orifices which are thus not of an aller gic nature In a previous publication (23) I pre sented seven cases of mucocutaneous syndrome in conjunction with primary herpes stomatitis

It is remarkable that no case yet appears to have been reported in conjunction with mononucleosis There are no less than 29 patients with this disease in the present material all were thus caused by in fection with Epstein Barr virus Cases after small pox vaccination have been observed earlier (4) In the present material as well there are three cases in which the syndrome was caused by vaccinia virus In one case it arose in conjunction with hepatitis B It is probable that there was a viral infection also in the 11 cases of serous meningitis even if this could not be confirmed virologically or serologically

Evidence that the agent in question was the caus ative factor lies in the circumstances that in 40 of 155 verified infections no drug was given before the onset of the syndrome There are untreated cases within all the aforesaid groups of infections In all cases of pneumococcal and urinary tract infections on the other hand provocative drugs have been given These bacteria probably lack or have a very slight antigenic potency

It is noteworthy in this context that no case of acute staphylococcal infection has been recorded A special form of febrile mucocutaneous syndrome Lyell s syndrome (10) is sometimes associated with this infection especially in children (11) Certain types of Staphylococcus aureus may form a toxin which produces a necrosis between epidermis and cutis (toxic epidermal necrolysis) without inflam matory reaction Nikolsky s phenomenon is posi tive Flabby thin blisters form and extensive areas of skin peal off (scalded skin syndrome) The pic ture is one of a second degree burn The syndrome can also be brought on however by roughly the same drugs which cause ordinary mucocutaneous syndrome I have seen this syndrome change to Lyell s type in one case of secondary staphylococ cal infection (24) The lethality is also high (about 25%) for this mucocutaneous syndrome in contrast to the ordinary type described here

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Pleuroperimyocarditis Caused by Immunization with Anticatarrh Vaccine

A Case Report

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ABSTRACT A case of pleuroperimyocarditis caused by immunization with anticatarrh vaccine is described During the most acute phase, circulating immune complexes were demonstrated in the patient's serum The possibility that these complexes represent a pathogenic mechanism in the illness and the value of anticatarrh vaccination are discussed

Bacterial anticatarrh vaccines have been used for almost 70 years for prevention of respiratory infections They are frequently used all over the world but although there are many articles dealing with possible preventive effects very little is known about their adverse effects We therefore wish to describe a patient who has been hyperimmunized with anticatarrh vaccine and developed signs of cardiac pleural and mild renal involvement probably caused by some pathological mechanism in which immune complexes are involved

CASE REPORT

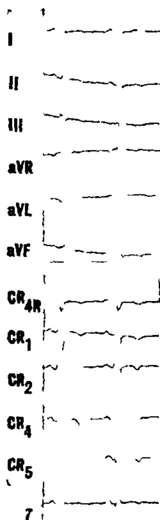
Examination

A 24 year-old technical student was admitted to the hospital in Nov 1974 with severe pains in the left lower part of his thorax and upper abdomen He also complained of intensive pain in his left shoulder region No heart murmurs pericardial or pleural friction sounds were heard Pulse 105 temperature normal and BP 125/80 There were no clinical or subjective signs of respiratory tract infection His breathing was superficial and he complained of increased pains when deep-breathing He had received repeated injections of a commercially available anticatarrh vaccine since Nov 1973 (Parke Davies) and also doses of highly diluted staphylococcal vaccine (National Bacteriological Laboratory Sweden about 3000 bacteria/ml) because of frequent upper respiratory tract infections The anticatarrh vaccine was administered in weekly subcutaneous doses of 0 25 0 5 0 5 and 1 0 ml respectively during the first month and thereafter in a monthly dose of 1 0 ml Staphylococcal vaccination followed the same schedule and was given in the same volumes Except for frequent upper respiratory infections the patient had always been in excellent physical and mental condition and is an active sportsman

Clinical progress

The patient s current history began 6 months after the start of immunization i e when he had received a total of 10 injections Approximately 14 days after the last one he experienced intensive precordial pain a general feeling of illness and an inability to perform even easy physical activities Most of these symptoms disappeared only to return again about 14 days after the next injection He described them as identical to those present on admission ECG showed slight ST elevations in most leads On the third day his temperature increased to above 38 C and on the next day there was a generalized T wave inversion (Fig 1) No pericardial or pleural friction rubs were heard Chest X ray showed an enlarged heart (Fig 2) interpreted by the roentgenologist as caused by pericardial effusion and also pleural fluid Echocardiographic examination confirmed fluid in the pericardial sac His temperature rose during the following two days to a maximum of 39 2 C

The laboratory investigations on admission showed normal Hb ESR and WBC ESR increased during the next two days to 32 mm/h and was 63 mm/h after one week The WBC also increased and reached its peak 12 100/mm³ on the second day A slight relative increase in the number of monocytes and neutrophils was found S ASAT and S ALAT were normal during the whole course of illness On the second day he also developed signs of renal involvement with proteinuria and increased WBC in urine On the same day circulating immune complexes were found in his serum The pathological findings in urine remained for the next three days His chest pains



F g 1 ECG sho ৷ ng general zed T wave invers n

persisted for about one week with gradual improvement and h s temperature fell markedly to normal values on the th rd day The T wave invers on returned gradually but was not completely normal unt l after about 14 days In tially because bacter al infect on could not be excluded p vamp cill n was given orally in da ly d ses of 1 05 g S nce h s case history trongly suggested an immunopath ol ↄg cal react n he was als g en a daily do e of 100 mg and methac n re tally Chest X ray contr l after e ght days showed complete d sappearan e of the pericard al and pleural effusion During the most acute two days f illness hi FCG was mon tored sh wing s nus rhythm all the t me The central ven ous pressure was normal A pleural puncture ৷ as performed and samples were taken for pathol ↄg al mmunolog al and m cr ↄb l g al n vest gat ns

M cr b l cs and mn un l cs

Several bacteri l ↄg al exam nat ns of sputum and urine showed negat ve results Cultures from nose naso-pharynx and pharynx showed n rmal flora The pleural exudate was sterile Complement f xat on tests n acute and convalescent sera aga nst influenza A and B adenovirus RS v rus orn th sis para nfluenza 1 2 3 Coxa kie B 5 echo 9 and Mycoplasma pneumon ae ga e no s gn ficant titre rise Search for Mycobacteria in the pleural exudate gave negat ve results Antistreptolys n antistaphylolys n and ant pneumolys n t tres were with n normal l m ts Complement fixat on test for H influenzae was sl ghtly increased 1 320 (upper l m t f r normal variat on 1 110) Normal IgG IgA IgM and IgE values Complement factor 3 sho ed a sl ght increase probably due to its act ng as an acute phase re tant Serum electrophores s had a typ cal acute react n pattern wh ch later l ke the C3 values returned to normal C4 value was normal No autoant bod es were dem nstrable w th immunofluorescence analysis Circulat ng immune com plexes occurred in serum (21) on the second day i e when the d sease was at ts peak w th h gh fever ren l in volvement etc The pleural fluid conta ned 94 mg/100 ml of C3 and 10 mg/100 ml of C4 The immune complex analyses were performed by Professor S E Svehag Un fortunately no test for activated complement pr ducts was performed but no free immune complexes were f und Complement fixat on test for N gonorrhoeae the syphi l t c react ons and the cryoglobul n test were negat ve

DISCUSSION

Studies of the importance of ant catarrh vaccine in the prevent on of respiratory tract infect on have been contrad ctory and sparse (1 2 5 7 8 16 19) So far no controlled study has been able to give a convincing p cture of such vacc nes cl n cal value This is very s rprising since evidently large quant ties of vaccines are used The amount used in Sweden during 1973 imported or manu factured in the country w s approximately 140 000 ml A large



F g 2 Chest X ray showing card ac enlargemen

variety of antigenic substances more or less well defined are present in the heat inactivated multi bacterial suspension constituting the vaccine as enzymes capsular or somatic components etc maybe also contaminants from the culture medium Repeated injections must greatly increase the pos sibility of certain unwanted immune reactions

Postvaccinal cardiac complications have been re ported after smallpox vaccination (14) although they seem to be rare There are also reports of complications when bacterial vaccines are used but with some exception they seem mostly to be mini mal and often negligible and few heart complica tions have been reported Severe adverse effects have however been reported both in man and ani mals e g amyloidosis in horses receiving intensive immunization with diphtheria toxin (23) and joint as well as cardiac valvular lesions in guinea pigs after immunization with polysaccarides from Klebsiella pneumoniae (12) In man repeated injections with pertussis vaccine have caused death in one case due to a diffuse vasculitis (3) and hyperimmuniza tion with streptococcal vaccine was the cause of amyloidosis in another (18) Recently Boulton Jones et al (4) described a case of glomerulo nephritis as the result of intense immunization with diphtheria pertussis and tetanus vaccine (DPT vaccine) Peeler et al (17) investigated the possible adverse consequences of intensive immunization in man by studying a group of laboratory workers who had been immunized with various antigens None of them showed evidence of clinical illness but had abnormal serum electrophoretic patterns ab-normalities of renal and liver functions and a high incidence of lymphocytosis

Our patient must according to his case history have had at least three attacks of pericarditis and/or pleuritis before hospitalization After his recovery he has had no further injections and is completely symptomless Kidney function is normal with a creatinine clearance of 130 ml/min his working capacity as studied by an exercise test is above the average and repeated resting ECGs over several months are absolutely normal Without a provoca tion test it is of course not possible to conclude for certain that the immunization with anticatarrh and antistaphylococcal vaccines was the cause of the patient s symptoms For ethical reasons such a test has not been performed However no other reasonable etiology has been found in our investiga tion

Repeated antigen injection is a well established technique for inducing immune complex nephritis in rabbits (6) and a similar mechanism probably exists in man The etiology of idiopathic pericarditis is obscure An immunological mechanism has been proposed in the postmyocardial infarction and the postpericardiotomy syndromes and antibodies to the myocardium have frequently been demon strated (9 11 13 15 20) The clinical significance of these antibodies is not clear but the study of Versey and Gabriel (22) is in accordance with an immune complex theory The development of mild reversible kidney involvement in our patient during his most acute illness his perimyocarditis and pleuritis as well as the occurrence of circulating immune complexes thus favour the suggestion of an immune complex mediated pathogenic mechanism This view is also supported by the finding of Hun der et al (10) that immune complexes are important in the pathogenesis of pericarditis associated with systemic lupus erythematosus It seems well estab-lished (4 6) that immunization creating immune complexes in antigen excess results in glomerulo-nephritis It is thus possible that the clinical symp toms of the patients are results of immune com plexes formed by antibodies and unknown antigens and it must again be emphasized that most of the antigens in the anticatarrh vaccine are characterized poorly or not at all

The importance of the staphylococcal vaccine in the present pathogenesis is difficult to evaluate but its low bacterial concentration makes it less likely as a causative agent We believe this case is illustra tive of complications that might be caused by inten sive anticatarrh vaccination The very widespread use of this type of vaccine the contradictory results *as to its value and the lack of information about* adverse effects urgently motivate large carefully controlled studies to elucidate its justification in the prevention of upper and lower respiratory tract in fections and the type and frequency of the adverse effects

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Pyoderma Gangrenosum Associated with Regional Enteritis

Improvement in Defective Granulocyte Function and Healing of Skin Lesions During Administration of Clofazimine

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ABSTRACT A defective uptake of oxygen by peripheral blood granulocytes during phagocytosis, indicating a subnormal phagocytic capacity, has been found in a patient with regional enteritis complicated by pyoderma gangrenosum (PG) During administration of clofazimine the granulocyte function normalized and the skin lesions healed It is possible that a defective granulocyte function may sometimes be involved in the pathogenesis of PG and that a clofazimine induced improvement in the function will favour healing of the lesions The result of treatment in our patient and in other cases recently published indicates that the drug may be worth trying in PG

Pyoderma gangrenosum (PG) is a progressive ulcerative disease of the skin which may complicate several chronic disorders e g ulcerative colitis (15) regional enteritis (1 15 16) and rheumatoid arthritis (17) The etiology and pathogenesis of the skin lesions remain obscure Treatment with corticosteroids will most often improve the condition (15) but sometimes very high doses are necessary (12 15) Recently a favourable effect of clofazimine a phenazine derivative has been demonstrated in patients with PG (12) The drug is active against Mycobacteria and is used in the treatment of leprosy (18) In addition clofazimine is known to improve the phagocytic activity of neutrophilic leucocytes (2 13) Macrophage function in vitro is also stimulated by clofazimine (3 4)

The present report deals with a case of regional enteritis complicated by PG There was an impairment of the granulocyte function which normalized during administration of clofazimine with a concomitant healing of the skin lesions

CASE REPORT

The patient is a 19 year-old woman In the summer of 1972 at the age of 16 she developed diarrhoea and colicky abdominal pains Gastrointestinal X rays disclosed widespread changes in the colon with pseudopolyposis and mucosal oedema The terminal ileum was narrowed The rectum had a normal appearance and the results of repeated sigmoidoscopic examinations and biopsies were normal She had a slight normochromic anaemia and ESR was 100 mm/h Although impossible to verify histologically the diagnosis of regional enteritis was made and she was treated with sulphasalazine Repeated X ray examinations have shown persistent changes in the colon and terminal ileum but her symptoms have gradually subsided

In Oct 1972 multiple itching pustules were observed symmetrically localized to her elbows and there were a few similar eruptions on her legs In Oct 1974 a necrotizing ulcer was noted on the left calf Despite local treatment the ulcer increased in size and was about 4 cm in diameter in Feb 1975 At this time another ulcer about 2 cm in diameter developed on her right calf (Fig 1*a*) A biopsy specimen revealed a purulent inflammatory reaction and an infiltration of lymphoid cells There were also signs of vasculitis and the biopsy findings were considered to support the clinical diagnosis of pyoderma gangrenosum

Treatment with clofazimine (Lampren® Ciba-Geigy) in a daily oral dose of 200 mg was given from March 25 to May 15 1975 The ulcer on the right calf healed completely with a thin pigmented scar and the ulcer on the left calf was considerably reduced The pustules disappeared during the treatment During a second course of treatment with clofazimine from June 15 to July 25 the lesion on the left calf also healed completely (Fig 1*b*) No recurrence of the ulcers was noted at a follow up two months after cessation of treatment

MATERIAL AND METHODS

Collection of leucocytes Venous blood was collected in heparinized glass tubes (Vacutainer Becton Dickinson France) and mixed with an equal volume of 2% Dextran

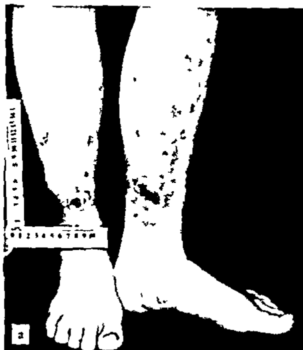
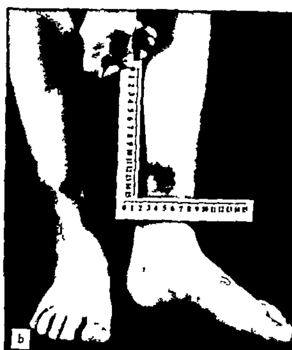



Fig 1 Skin lesions before treatment (*a*) and after two courses of treatment with clofazimine (*b*)

250 (Pharmacia Fine Chemicals Uppsala Sweden) in 0 15 M NaCl The tubes were left standing at room temperature for 30–45 min to let the erythrocytes sediment The leucocyte rich supernatant was withdrawn and centrifuged at $400 \times g$ for 5 min Remaining erythrocytes were haemolyzed in 0 87% ammonium chloride for 5 min The leucocytes were washed once in Ca free Krebs Ringer phosphate buffer (KRP) pH 7 4 and the concentration was adjusted to 10×10^6 granulocytes/ml This cell suspension was used in the oxygen consumption test

Oxygen consumption Oxygen consumption during phagocytosis was measured with a Clark electrode mounted in a 2 24 ml glass stoppered incubation chamber equipped with magnetic stirring and thermostatic heating to 37°C (Eschweiler & Co Kiel West Germany) A paper recorder was connected for continuous recording of the oxygen consumption

Heat killed yeast cells in a concentration of $5 \times 10^5/\mu l$ were preopsonized with pooled normal serum (3 parts serum + 5 parts yeast cell suspension) for 5 min Prewarmed leucocyte suspension (1 000 µl) was mixed with 800 µl of preopsonized yeast cell suspension and 700 µl KRP and the mixture transferred to the incubation chamber The chamber was closed and the recording started immediately The oxygen consumption was calculated according to the following formula (8)

$$\text{ml } O_2 = PO_2 \times \frac{\alpha}{760} \times V$$

α = absorption coefficient V = volume of the reaction chamber and expressed as µl $O_2/10^7$ gran /min

Leucocytes from healthy donors were run simultaneously as a control each time and the statistical calculations were based on the paired *t* test as described by Hoffman and Bullock (9) An oxygen consumption index (OI) was calculated

$$OI = \frac{O_2 \text{ cons patient} - O_2 \text{ cons control}}{\text{normal between subject S D}}$$

The dominator is equal to the S D of seven normals tested simultaneously The significance limits were $OI \leq -2\ 45\ p < 0\ 05$ $OI \leq -3\ 14$ $p < 0\ 02$ $OI \leq -3\ 71$ $p < 0\ 01$

RESULTS

The OI of the granulocytes was significantly subnormal −3 87 prior to treatment with clofazimine Seventeen days after the institution of therapy the OI had normalized −0 65 (Fig 2) When the patient had been off treatment for 3 weeks the OI was again subnormal −3 41 During the second course of treatment the OI was significantly increased to −1 96 (Fig 2) Two months after withdrawal of clofazimine the OI was within a normal range −0 71

DISCUSSION

Although the etiology and pathogenesis of PG are unknown several abnormalities in immune re

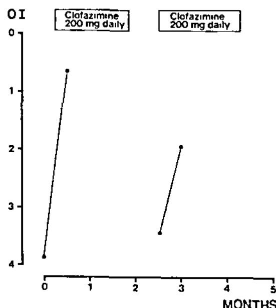


Fig 2 Oxygen consumption indices (*OI*) of phagocytizing granulocytes before and during administration of clofazimine

sponses have been suggested to be of importance for the development of the skin lesions Hypogammaglobulinaemia has been demonstrated in some patients (10) and abnormal serum proteins have been found in others (5) An impaired cell mediated immunity may occur in PG (7) but it has also been pointed out that the skin lesions may have traits in common with a cell mediated skin graft rejection (6) Such divergent findings and the present demonstration of a defective granulocyte function associated with PG indicate that various abnormalities of humoral and cellular defence mechanisms may be found in PG and that the pathogenesis of the skin lesions may be heterogeneous

Oxygen consumption rate during phagocytosis was chosen as a sensitive measure of granulocyte function Resting granulocytes have a very low oxygen consumption but increase this function considerably during phagocytosis The oxygen consumed is almost quantitatively converted to hydrogen peroxide due to oxidase activity (19) Since the oxygen consumption rate correlates to phagocytosis (14) it is evident that quantification of the oxygen consumption rate during phagocytosis is a highly adequate measure of granulocyte function The increase in phagocytic activity during the administration of clofazimine was repeatedly demonstrated in our patient and confirms previous findings of a stimulatory effect on granulocyte function by the drug (2 13)

It is interesting to note that the drug induced normalization of the granulocyte function was associated with healing of the skin lesions Michaelsson et al (12) noted a slightly reduced phagocytic capacity of neutrophilic leucocytes in some patients with PG but the finding was not consistent By using a highly sensitive method to test the phagocytic capacity it was possible to demonstrate an impaired granulocyte function in our patient It remains to be clarified whether this abnormality is common in patients with PG Moreover the possible role of an impaired granulocyte function in the pathogenesis of PG is unknown It is however possible that a defective function of phagocytic cells may sometimes be of importance for the development of PG and that an improvement in phagocytosis may be beneficial in some cases It is of interest in this context to note that a clofazimine induced improvement in a subnormal phagocytic activity of the granulocytes in patients with pustulosis palmaris et plantaris has been found to be associated with a clinical improvement in the disorder (13)

Michaelsson et al (12) have recently demonstrated a positive effect of clofazimine in eight patients with PG The results of treatment in their patients and in the present case suggest that the drug may be worth trying in PG

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EDITORIAL

The Essence of the Clinician's Art

In the last ten or fifteen years studies of the art of being a physician have begun to emerge The study of *how* doctors do *what* they do has moved from the stage of history or anecdote into the field of computers operational analysis and theories on games and decision making (3)

My interest in this area was aroused in the early 1960s when the computer came to the clinic and when vague concepts and delayed decisions had to be boiled down to yes no or do not know (6) It then occurred to me that never during my studies or my training had anybody taught me really how we do what we do There were rules of thumb there were options and there was experience and intuition but how all this worked in practice was rather uncertain At first my interest was attracted to what might be labelled the physiology of diagnostics (2) Later I tried to break down the whole procedure of handling a clinical case from the point of view of the medical record the document that should record our *findings* our *hypotheses*—formulated or hidden in the search for clues and confirmations—and our consequent *actions* their *results* and the *guidelines* for *the future* (4)

But it soon became apparent that centering one s attention on the medical record was not enough or even might lead astray Because the physician s concept of a patient and his diseases derives from an integration in his own mind of the *real* patient in front of him and the *artificial* one embodied in the collection of medical records from various periods of his life (5) Over the last ten–twenty years medical records have improved in detail and volume but whether they are now more precise and more relevant is sometimes doubtful At least it has been one of the duties of a head of department brought up on the traditional teaching of medicine to warn his students and younger colleagues not to desert the real patient and the bedside for the popular escapism of the sitting round a committee work performed away from the proper subject of the study and with the usual dispersion of responsibility that goes with most committee work

Yet there is definitely a case for sober studies on medical decision making Such studies can have at least three starting points One is that of the computernick trying to discern in a tremendous number of incomparable data some common denominators some recognizable patterns that might throw light on what is really going on Another comes from the departments of psychology and the like which long ago gave up dealing with what people think they are doing and should be doing and devote themselves instead to an imaginary world of models —so why not think out a model of what goes on in the physician s brain? A third albeit humble approach is to follow the advice of the Greek philosopher who 2300 years ago remarked that man is the proper measure of everything (including man himself) Thus studying how we do what we do (or should do)—critical studies by outside observers yet in contact with their subjects for the proper interpretation of the whys?" —might eventually shed some informed light on medical decision making This empirical avenue is one that some of my junior collaborators and I have tried to explore (6 9) in order to provide material for future studies by more sophisticated methods

There are at least three problems with decision making in internal medicine First most models or rules of thumb or programs assume that one has to deal with only one disease (or syndrome) In our field we know that this is mostly not the case Second we are often pressed for time not only is there for us as there is in acute surgery often a deadline before which something simply *has* to be done but there is also rarely a once and for all decision to be made diagnostically and a consequent therapeutic regimen to be applied On the contrary we have to make adjust or reverse our decisions all the time as we follow the patient In short a

study of medical decision making has to proceed along a time axis and preferably combine this with a dig into fundamentals beneath what appears to be the surface layer where action can be identified Third and not least complications are liable to arise en route spontaneously or in response to interventions One such story is told in a case report in this issue (8) partly in response to the plea by the Editor in-chief in his recent editorial (20)

Yet this should not detract from serious attempts at uncovering the basic patterns in medical decision making for it is clear that by scientific scrutiny of our habits techniques and programs many fallacies will be detected many irrelevant procedures discarded and the minds of an increasing number in our own profession could be tuned in to put the proper and relevant questions not only to their patients but—alas!—to themselves Studies in and of medical decision making are to be regarded as an audit of our performance and as such to be welcomed But they have to be made by people who know what doctoring means and who realize that the essence of the clinician s art actually is to *make decisions* a tremendous number every day often on the basis of insufficient evidence under the pressure of time and/or finance (including in some countries malpractice suits) and to make them with (at least outwardly) the appearance of a calm dedicated and warmly human personality

But you cannot make decisions or better advise the patient about his options unless you know the *prognosis* under a given set of conditions That is where mathematics comes into the process and this is also the field in which a growing number of publications mainly from England the USA and France is now forthcoming (1 10 11 13–19) More is to be expected when proceedings are published from some conferences on the subject such as those at the Royal College of Physicians in London and a recent meeting of the International Federations of Information Processing in Dijon France

It is already evident that there are useful methods for expressing prognosis under given conditions in mathematical terms—provided suitable case materials can be collected in ways that lend themselves to the appropriate mathematical methods forming data bases to be fed into computers Prognostic data should be collected both retrospectively and prospectively—the study of betting —in order to procure information about the validity of true prognostication Studies of the latter type are presently being tested (7, 12) Eventually such endeavours will result in acceptable programs as guidelines for the physician s own decision making and for the advice he can give patients, when an informed public increasingly seems to demand the right to take fate into its own hands

Finally a plea for a greater understanding and acceptance by the medical profession itself—and its editors!—of papers dealing with questions of this kind We know from experience that it is hard to find room for basic presentations in this area in medical journals at home and abroad Editors feel that they are too complicated for—or too alien to —the medical profession which looks with disdain at formulas containing letters instead of figures and at examples which often appear to be either too naive or too abstract to be recognized by the physician as belonging to his day to-day activity In other cases the mathematical or computer apparatus necessary for solving rather simple problems seems to be either outside the reach of most physicians or unnecessarily complicated for the immediate purpose All this can be said But unless studies in this field are incorporated in the ordinary technology of medicine they will be relegated to a separate area which is unlikely to benefit anybody It is for this reason that this editorial has been written in simple language—the one we as clinicians use among ourselves—in order to introduce the readers of this journal to a rapidly developing area on which more information can be collected by reading some of the more fundamental—or more recent—international papers referred to in the text

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An Unusual Case of Quinidine-induced Systemic Disease as an Exercise in Clinical Decision-making

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On Jan 12 1976 a male patient 57 years old was referred to one of us (G B) from a general practitioner because of an ECG showing ventricular arrhythmias and a history of vertigo It was decided to observe the patient in our Coronary Care Unit (CCU) as he was shown to have series of ventricular ectopic beats (VEB) and occasional short runs of ventricular tachycardia

The patient s history revealed an episode of rheumatic fever in childhood which apparently had left no sequelae in the form of valvular heart disease Apart from this there was nothing remarkable in his previous history The course of events during his present illness will be reported in the form of a running history in order to describe the difficulties inherent in diagnosis and treatment of obscure cardiac illness—an exercise in medical decision making

COURSE OF EVENTS

Previous history

Childhood age 9 Rheumatic fever Since then in perfect health sportive reserve officer tennis player

Present illness

Fall 1975 Upper respiratory infection for 2 weeks (no antibiotics) tiredness Dec 1975 Attacks of vertigo and palpitation No cardiac pain no dyspnoea General practitioner Laboratory tests normal Jan 1976 ECG *ventricular arrhythmia* Admitted to our Department of Medicine CCU

Jan 12 1976

Findings on admission Physical examination Heart normal sounds no murmurs BP 140/70 Lungs normal liver not enlarged X ray Heart volume 530 ml/m² BSA Lungs without remark ECG Periodically bouts of VEBs and fusion beats at times rate 120/min=VT No ST T changes Minor right intraventricular conduction defect Laboratory ESR 3 mm/h WBC 5500 ASAT ALAT CPK alkaline phosphatases normal Temperature afebrile

DECISION 1

Considered to be ventricular tachyarrhythmia without known cause Possibly postinfectious subacute myocarditis? Atherosclerotic heart disease? Slight cardiac enlargement at X ray interpreted as possible athlete s heart

Programme

Oscilloscopic observation (later on by means of telemetry) Chemical and microbiological tests for infectious diseases thyroid function immunological activity

Drug treatment (Jan 13)

Seloken® (metoprolol) 50 mg×2 After just a few hours reduction of VEBs with return of stable sinus rhythm on Jan 13 Exercise ECG on the next day shows return of VEBs during exercise at 130 W No ST depressions No pain

DECISION 2

Jan 14-18

Because of the persistent tendency to VEBs during work it was deemed desirable to add another antiarrhythmic drug Therefore sustained release *quinidine* in a dosage of 0 4 g twice daily was added to previous treatment The patient feels well and shows no signs of heart failure Telemetry shows no abnormalities

Jan 19

After a stepwise increased exercise test to 230 W with normal heart rate response and no arrhythmias the pa

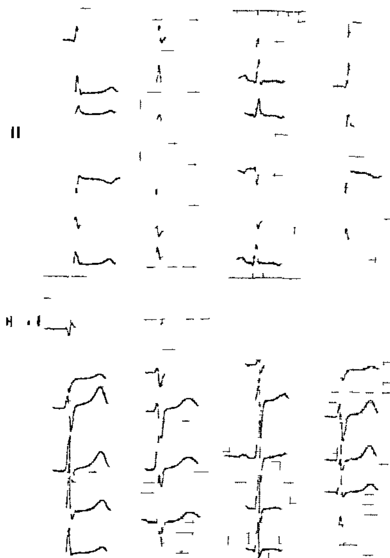


Fig 1 A normal ECG was found on Jan 13 ST T changes were noted on Feb 6 and 10 which then dis appeared by Feb 16

tient was allowed to leave the hospital Medication at home 50 mg Seloken* twice daily+0 4 g quinidine twice daily

Jan 23

Reports back for control ECG essentially normal at extra long tracing Heart rate 50/min Feeling well working half-day Allowed to go to his country place and report back in 2 weeks before further decision on return to professional activities

Feb 3

Reports back *ambulatory* No objective symptoms from the heart no palpitations but feeling unusually tired in the afternoons and occasionally a little uneasy ECG does not show any arrhythmias but the ST region differs from previous ECGs (Fig 1) This is interpreted as a possible effect of quinidine The question is now *Is the pat ent tired from his disease or from his treatment?* Could this be an effect of a β blocker on a person with latent cardiac failure? Physical examination nothing noteworthy observed

DECISION 3

Of the drugs given the β blocker is most likely to induce myocardial failure (if such were the case) Therefore as a first step reduce the dose of β blocker to 50 mg/day Check *chest X rav* be cause of the earlier mild enlargement Make *echocardiogram* to study cardiac contractility

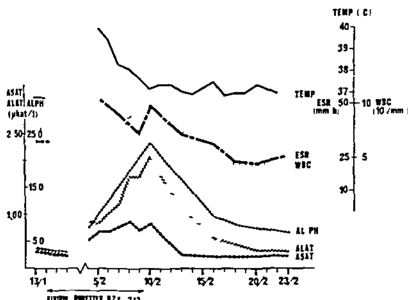


Fig 2 Some laboratory data during the acute phase

Results

Chest X ray Heart size not significantly increased since Jan 12 but clear signs of left ventricular failure in the form of widened pulmonary vascular structures and redistribution of the pulmonary blood flow Some interstitial edema around the bronchi vascular structures and in the interlobar spaces atelectasis at the lung bases

Echocardiogram revealed left ventricular dilatation without hypertrophy normal aortic and mitral valves and possibly a hypokinetic area in the lateral part of the left ventricle The estimated ejection fraction (35%) and cardiac output (3.5 l/min) were both low indicating myocardial failure

DECISION 4

Feb 4

Obviously the patient s subjective symptoms are due to manifest left ventricular failure The patient is *readmitted* for further study under telemetric observation of the ECG with the intention of withdrawing the β-blocker completely and possibly digitalizing the patient with due consideration of a possible reappearance of the ventricular arrhythmia At physical examination the patient shows no conventional signs of cardiac failure Regular rhythm heart rate 64 BP 120/70 Seloken is withdrawn completely

Feb 5

On this day the patient runs a temperature of 39 2°C a heart rate of 75 an ESR of 56 mm/h WBC 4 700 He admits that he may have had some fever also some days before During the night sinus rhythm with a few VEBs but in the morning the patient develops atrial flutter with a ventricular rate of 130/min

DECISION 5

The patient is transferred back to the CCU The flutter reverts to sinus rhythm with VEBs single and multifocal after 40 mg furosemide and 0 37 mg g strophanthin i v

DECISION 6

It is now realized that the patient is deteriorating with regard to his pump function possibly under the added strain of a high temperature In view of the low WBC the febrile condition is assumed to be due to a virus infection particularly as an epidemic of influenza A (Victoria) has been identified in the city All efforts are aimed at supporting the failing myocardium

Feb 6

The patient is treated in the CCU with digoxin 0 25 mg furosemide 40 mg and Moduretic® (50 mg hydrochlorothiazide+5 mg amiloride) in addition to quinidine The high temperature continues without local symptoms and with a low WBC (Fig 2) Quinidine concentration 3 0 μg/ml ECG still shows ST T changes in the lateral precordial leads

Feb 7

The temperature is 38 2°C The patient is rather tired but not in manifest failure There are still no symptoms of upper respiratory illness

Feb 8

Recurrence of atrial fibrillation and flutter with a ventricular rate of 130–150/min BP 115/70 Lungs For the first time basal rales Temperature 37.5°C Liver not enlarged

no signs of venous thrombosis After 4 ml furosemide i v reversion to sinus rhythm Furosemide 80 mg/day is prescribed and Moduretic* withdrawn

A series of laboratory tests (Fig 2) now makes it clear that the patient in addition to the elevation of his ESR has *developed markedly elevated liver enzyme values* The prothrombin index has fallen from 87 to 50 The WBC is within the upper normal range

The question arises whether these liver tests indicate right ventricular failure or something else The suspicion is entertained that the quinidine might be the culprit

ANALYSIS OF THE SITUATION

Feb 9

Summary During the late fall gradual development of myocardial disease manifesting itself in ventricular arrhythmias tiredness and occasional light headedness but no symptoms of angina pectoris This could—in retrospect—be related to an upper respiratory infection earlier in the fall

After treatment with a β blocker and quinidine improvement of the arrhythmia but development of cardiac failure fever without leukocytosis and pathological liver tests After withdrawal of β blocker reappearance of arrhythmias now also supraventricular but no improvement of cardiac failure or liver tests

INTERPRETATION

Even though the temperature and relative leukopenia may be interpreted as due to a possible virus infection (however without other influenza symptoms) and the abnormal liver tests attributed either to a virus disease or to a right heart failure the possibility that the other antiarrhythmic agent—*quinidine*—might be responsible must be thoroughly investigated Among other conditions that might explain the basic illness immunological disease (collagenosis) may be a possibility as well as right heart failure secondary to pulmonary embolism

DECISION 7

1) Investigate literature concerning side effects of quinidine and pending this *withdraw the drug* 2) Speed up if possible laboratory answers to questions of *virus* and *immunity* 3) Blood gas analysis and lung scintigram to study the possibility of acute (or chronic) pulmonary embolism 4) Arterial *blood cultures* (old rheumatic fever—possible SBE)

Results

1) First enquiry to our Laboratory of Clinical Pharmacology gives only the conventional quinidine side-effects (6) 2) Viral and immunological tests negative 3) Arterial $PO_2$62 $PCO_2$27 which lend some support to the possibility of pulmonary embolism 4) Blood cultures negative

DECISION 8

1) Instruct the Laboratory of Clinical Pharmacology to make an *extensive* literature search 2) Order a pulmonary scintigram

Results

Seven cases of quinidine induced liver disease had been reported during the last few years (2–5 7–9) In three patients granulomatous hepatitis was diagnosed on liver biopsy (2 3 7) whereas the association between quinidine and liver injury was verified by means of a provocation test in four patients (2 4 5 7) The salient features of the quinidine induced liver damage are fever elevation of serum concentrations of GOT (ASAT) GPT (ALAT) LDH and alkaline phosphatase *Concomitant* rises in serum bilirubin (4 7 9) and prothrombin time (4) are also reported All values return to normal levels after withdrawal of the drug

Feb 10

The patient is improving slightly Temperature 37.2°C *WBC* 9900 *Lung scintigram* No signs of pulmonary embolism *Chest X ray* Further improvement of the interstitial lung stasis *ECG* Sinus rhythm ST T deformities including some T wave inversion corresponding to the lateral and diaphragmatic walls *Echocardiogram* No noteworthy changes since Feb 3 Both ventricles dilated but no signs of right ventricular strain in the septal activity Still low ejection fraction and cardiac output *Telemetry* Regular sinus rhythm with a few monofocal late VEBs At the slightest effort however the heart rate increases from 90 to 130

Feb 11

The patient is now treated with digoxin 0.25 mg furosemide 40 mg×2 and further clinical improvement is seen However telemetry shows increased frequency of ventricular arrhythmias including ventricular tachycardia *Ballistocardiogram* The ratio between the IJ amplitude and the IJ duration is very low which corresponds to severe myocardial disease

DECISION 9

Feb 12–23

Metoprolol 50 mg×2 is reinstituted Continuing improvement subjectively and laboratory wise (Fig 2) Serum bilirubin and prothrombin index now within normal range

Chest X ray (Feb 16) Heart volume 350 ml/m² BSA slight redistribution of the pulmonary blood flow On Feb

23 heart volume 340 ml/m² normal pulmonary blood flow

ECGs Feb 12 T wave inversions in leads II IIIaVF V_{5-7} Feb 13 ST T segments now slightly generally depressed No arrhythmias Feb 16 and Feb 20 No arrhythmias Remaining slight ST T depressions in precordial leads only Feb 23 ECG normal Exercise ECG up to 110 W reveals no arrhythmias on Feb 20 No augmentation of ST T depressions

Echocardiogram Decreased heart rate to 54 (effect of metoprolol) Improved ventricular performance with an ejection fraction of 60% and cardiac output of 4 8 l/min The contraction pattern is generally better including the area in the lateral wall

Ballistocardiogram The IJ amplitude has increased by 50% though the heart rate has decreased The net effect is improved The IJ duration is still delayed with an abnormal pattern compatible with surpassed myocardial damage

DECISION 10

Feb 24

The course of events seems to follow the pattern of a hypersensitivity quinidine reaction recently described It is decided not to perform a liver biopsy as the evidence seems sufficiently convincing and the patient may be considered to have suffered enough inconvenience already Neither is it considered warranted to expose him to quinidine once more in order to prove the case On the contrary the patient is instructed never to take quinidine again

The patient is released with digitalis furosemide and metoprolol as above and instructed to report back in a week for further control

March 3

Ambulatory control Feeling well Physical examination without remark Heart rate 48 BP 130/80 All liver tests normal ESR still somewhat elevated No VEBs on ECG ST T normal

DECISION 11

Reduction of metoprolol to 0 50+0 25 mg Furosemide conditionally withdrawn

COMMENTS

It appears highly probable that this case represents another instance of *quinidine* hypersensitivity Previous reports have dealt almost exclusively with the liver damage as such In our patient one cannot escape the impression that the damage may have affected the *myocardium* too turning a latent myocardial insufficiency into a manifest failure It could furthermore well be that some of the radiological findings in the lungs independently of the myocardial affection belong to the picture as they may do in other examples of drug hypersensitivity

This case also raises the question whether among the millions of patients all over the world who are being treated with quinidine a deterioration of their condition due to prolonged quinidine exposure may in an unknown number of cases be interpreted erroneously as due to the underlying *disease* rather than to an ingredient in its treatment It is for this reason we have chosen to describe this case—the first in Sweden and the second in Scandinavia as far as we know—in the terms of medical decision making to point out the difficulties obstructions and uncertainties that are inherent in our handling of the unexpected

As to the cause of the cardiac disease that initially brought this patient to hospital we are still as uncertain as before In view of the reduction in heart volume it now seems probable that the moderate cardiac enlargement observed already on the first hospital admission cannot be dismissed as a benign athlete s heart but reasonably represented a cardiac dilatation due to myocardial disease and latent failure either concomitant with or due to his ventricular arrhythmia

ADDENDUM NOV 1976

The patient is in very good condition and has resumed his work and tennis playing No arrhythmia is observed on the ECG His medication consists of digoxin 0 25 mg and metoprolol 25 mg×2 Laboratory tests including ESR WBC ASAT ALAT and alkaline phosphatase have remained normal

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Prolonged Induction of Germfree Bile Acid Pattern in Conventional Rats by Antibiotics

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ABSTRACT Male conventional rats have been treated for five days with benzylpenicillin, neomycin kanamycin, erythromycin bacitracin+neomycin, succinylsulfathiazole or metronidazole Total fecal bile acids were analyzed in samples collected during periods of three days during the pretreatment period and during the eight weeks following drug treatment Metronidazole or succinylsulfathiazole had no or minor effects on the conventional bile acid pattern and the ' bile acid index ' (ratio β-muricholic acid/deoxycholic acid) remained low Benzylpenicillin, neomycin or kanamycin induced a germfree bile acid pattern, i e increased the relative amounts of α and β muricholic acid in feces and eliminated deoxycholic acid and hyodeoxycholic acid from feces The high bile acid index was normalized within three weeks after termination of drug treatment but the excretion of α and β muricholic acid was not normalized until a normal flora had been established by giving an enema with intestinal contents from *intact, conventional* rats Treatment with erythromycin or bacitracin+neomycin also produced a germfree bile acid pattern In these cases, the bile acid index was not back to normal until after five to eight weeks and the excretion of the muricholic acids was not normalized until an enema with intestinal bacteria had been given It is suggested that these long lasting effects of antibiotics on the metabolism of bile acids in the intestinal tract should be considered after short term antibiotic therapy in humans

Recent studies in our laboratory have shown that certain germfree characteristics such as proteolytic activity in the feces (1) the electrophoretic pattern of the supernatant of feces (6) and the absence of coprostanol (2 5) and stercobilin formation (8) are produced in conventional animals by feeding with antibiotics (7) In view of the well known effects of sulfa drugs and antibiotics on the pattern of bile acids in intestinal contents of conventional rats (10 11) it was considered of interest to study the duration of the germfree pattern of fecal bile acids in conventional rats following feeding with antibiotics

MATERIAL AND METHODS

Male conventional rats of the Swedish AGUS strain weighing about 275 g were housed individually in metabolism cages (This rat strain of Long Evans origin has been reared under germfree conditions at the Department since 1956 It has later been established at Laboratory Animal Center Carshalton England and labelled AGUS) The personnel did not practice any isolation or aseptic measures The animals were fed an autoclaved semisynthetic diet with 10% arachis oil as source of fat (3) and water ad lib After a pretreatment period of 5 days doses of antimicrobial drugs were dispersed in 1 ml aliquots of water and given by stomach tube once a day for 5 days The following substances were used in the given doses per 24 h benzylpenicillin (Bensylpenicillin Kabi) 360 000 IU/kg b wt neomycin (Neomycin Upjohn) 180 mg/kg b wt kanamycin (Kanamycin Ferrosan) 35 mg/kg b wt erythromycin (Abbocin® Abbott) 360 mg/kg b wt bacitracin+neomycin (Bacimycin A L) 27 000 IU and 550 mg/kg b wt respectively succinylsulfathiazole (Sulfadigesin Astra) 360 mg/kg b wt. and metronidazole (Flagyl® Leo) 20 or 100 mg/kg b wt. For each compound 2 rats were used 2 rats given water only served as controls Eight weeks after the treatment had been initiated each animal was given an enema with 1 ml of a 10^{-1} dilution of cecum contents from 6 intact conventional rats of the same strain

Feces were collected daily for 72 h with an interval of four days between each period of collection and stored at −20°C until analyzed Feces from a 72 h period were weighed and homogenized in 50 ml ethanol The homogenate was refluxed for 2 h in chloroform/methanol 1 1 (v/v) and the extract was filtered through glass wool and pooled with the ethanol extract After evaporation in vacuo the extract was dissolved in 70% (v/v) ethanol and extracted with hexane The ethanol phase was then evaporated in vacuo and dissolved in methanol

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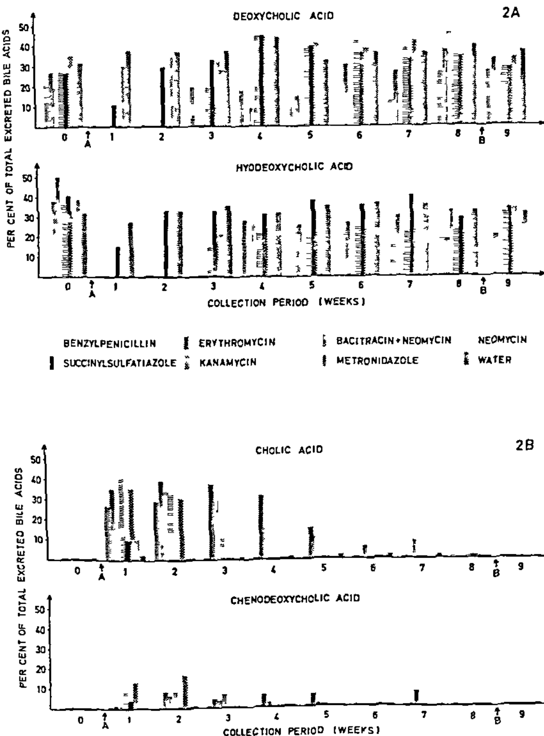
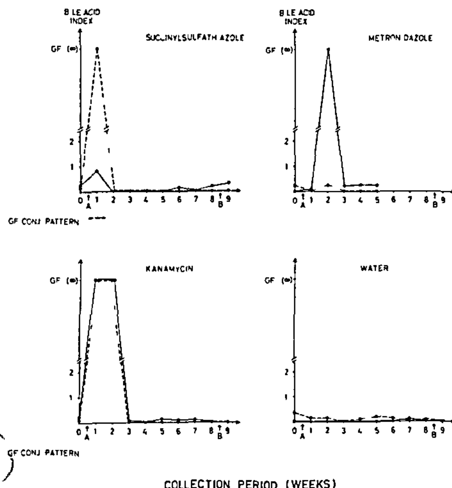


Fig 2 Relative amounts of deoxycholic acid and hyodeoxycholic acid (2A) cholic acid and chenodeoxycholic acid (2B) and α and β muricholic acid (2C) in feces from rats following treatment with benzylpenicillin erythromycin bacitracin+neomycin neomycin succinylsulfathiazole kanamycin metronidazole (20 mg/kg) and water (at time *A*) and following administration of an enema with cecal contents from intact rats (at time *B*)



perimental animals about five weeks after treatment with antibiotics judging from the reappearance of deoxycholic acid and hyodeoxycholic acid in feces

Bile acids are physiologically important compounds that have been implicated in the etiology of for example atherosclerosis and gastrointestinal disorders The present findings showing that short term treatment with antibiotics results in long lasting disturbances of the intestinal bile acid metabolism in rats may have to be taken into consideration in discussions on antibiotic therapy in humans

ACKNOWLEDGEMENTS

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Studies on Plasma Lipid and Phospholipid Composition in Pernicious Anemia before and after Specific Treatment

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ABSTRACT Patients with pernicious anemia (PA) have been compared with a reference group concerning the concentration of lipids in plasma before (34 cases) and after (15 cases) treatment with vitamin B_{12} The lipid parameters in plasma and in postheparin plasma have been measured before and after 6 hours' incubation at 37°C before and after specific treatment In a limited number of cases the lecithin cholesterol acyl transfer (LCAT) rate has been determined In relapse, the PA cases showed increased free fatty acid (FFA) and triglyceride (TG) concentrations but decreased concentrations of total cholesterol (TC), unesterified cholesterol (UC) and all examined phospholipid fractions After treatment, FFA remained unchanged and TG decreased while TC and possibly also UC and the phospholipid fractions were in line with reference levels After incubations, UC and phosphatidylcholine (PC) decreased and lysolecithin (LL) increased FFA increased and TG decreased Incubation of postheparin plasma resulted in an augmented decrease in TG and PC and increase in FFA and LL In relapse the changes on incubation were less pronounced than after treatment The LCAT rate was low but within the normal range before treatment in the six cases examined After treatment LCAT rates increased but were still normal in relation to the plasma lipid concentrations The study showed a decreased net esterification of cholesterol and LL formation on incubation of plasma in PA cases in relapse This finding might be explained by the low concentration of plasma substrates for the LCAT reaction and phospholipases After treatment the concentration of lipid substrates was restored to normal, with subsequent normal LL formation and cholesterol esterification The observations could also explain the frequent lack of stabilization of erythrocytes suspended in plasma of PA after incubation resulting in virtually the same ESR after as before this procedure Due to low LL formation following the insufficient substrate availability for LCAT and phospholipase, the previously reported critical level of the LL concentration is not reached and cannot achieve its normal reduction of the ESR

In the early forties Berlin (1 2 3) reported that the normal heat stabilization of the red cells suspended in incubated plasma was absent in cases of pernicious anemia (PA) in relapse It was suggested that this might be due to a considerable decrease in lysolecithin in the plasma of PA cases and that the increase in this compound on incubation was definitely less than in normal plasma It was concluded that this was probably due to absence of or a decrease in the activity of lecithinase normally present Later it was shown (5 6 7) that heparin injected in small amounts increased the normal lysolecithin formation during incubation in normal as well as in PA cases

The demonstration of a lecithin cholesterol acyl transferase (LCAT) activity as an explanation of the postincubation suspension stability of red cells and a decreased LCAT activity in cases with diminished or absent heat stabilization of the erythrocytes was reported by Scherer and Ruhenstroth Bauer (9) Likewise Miller and Thompson (8) recently found that in cases of PA (as well as in malabsorption cases with hypocholesterolemia) the LCAT activity was markedly reduced After treatment with vitamin B_{12} LCAT activity increased significantly At the same time both free and esterified cholesterol fractions increased with only a minor change in the free esterified cholesterol ratio These workers concluded that the limiting factor for LCAT activity in these cases was probably the amount of the specific substrate

Table I *Lipid and phospholipid concentrations (mmol/l) in normal subjects and patients with pernicious anemia (PA) before and after vitamin B_{12} treatment*

FFA=free fatty acids TG=triglycerides TC=total cholesterol UC=unesterified cholesterol PL=total phospholipids LL=lysolecithin (=lysophosphatidylcholine) SP=sphingomyelin PC=phosphatidylcholine (=lecithin) PE=phosphatidylethanolamine

	FFA	TG	TC	UC	PL	LL	SP	PC	PE
1 *Normal subjects*									
Mean	0 539	0 90	5 85	1 83	3 45	0 203	0 748	2 45	0 043
S D	0 068	0 38	0 51	0 27	0 18	0 010	0 050	0 13	0 010
n	20	27	27	17	27	27	27	27	27
2 *PA in relapse*									
Mean	0 755	1 66	4 07	1 26	2 36	0 134	0 490	1 70	0 043
S D	0 307	0 73	1 35	0 41	0 54	0 052	0 148	0 37	0 026
n	31	34	34	30	34	34	34	34	34
3 *PA in remission*									
Mean	0 765	1 36	5 67	1 62	3 32	0 182	0 700	2 37	0 075
S D	0 252	0 54	1 64	0 57	0 82	0 052	0 192	0 59	0 042
n	14	15	15	14	15	15	15	15	15
Mean difference[a]									
2–1	+0 216**	+0 76***	−1 79***	−0 57***	−1 09***	−0 069***	−0 258***	−0 75***	0 00
3–2	−0 027	−0 50**	+1 11**	+0 23	+0 77**	+0 041**	+0 161**	+0 54**	+0 03**
3–1	+0 226**	+0 47**	−0 18	−0 20	−0 13	−0 02	−0 05	−0 08	+0 03

[a] Tested by *t* tests of paired (3–2) and unpaired (2–1 and 3–1) groups **p*<0 05 ***p*<0 01 ****p*<0 001

available i e free cholesterol and lecithin rather than enzyme deficiency

We have collected a fairly large material of PA cases over several years with extensive investigation of several lipid and phospholipid parameters before and after specific treatment In a limited number of cases we have followed the changes in LCAT activity

MATERIAL AND METHODS

Thirty four cases of classical PA were examined The diagnosis was confirmed by means of conventional clinical and laboratory procedures including bone marrow examination serum B_{12} determinations and normal response to specific treatment Fifteen cases were reexamined two weeks to four months after the initiation of vitamin B_{12} therapy Our normal material published earlier (5) served as controls excluding the age group young men

Blood was drawn after an overnight fast into heparin tubes which were immediately cooled in ice Plasma was separated by centrifugation at +4 C Concentrations of individual phospholipids i e phosphatidylcholine (PC) lysolecithin (LL) sphingomyelin (SP) and phosphatidyl ethanolamine (PE) and concentrations of triglycerides (TG) free fatty acids (FFA) total cholesterol (TC) and unesterified cholesterol (UC) in plasma were determined before and after 6 hours incubation at 37°C and before and after B_{12} treatment as previously described (5 10) In 17 cases the same determinations were made before treatment and after i v injection of 5 000 IU heparin and blood samples were drawn after 10 min In 6 of these subjects postheparin plasma was collected also after specific treatment

Since the analysis of this material was concluded the high density lipoprotein (HDL) lipid concentration and the rate of lecithin cholesterol acyl transfer have been analysed in 6 PA cases before and in 4 of them also after B_{12} treatment The methods and the reference material used were recently presented in detail (11 12)

Conventional statistical methods have been used throughout the investigation

RESULTS

PA cases in relapse showed increased concentrations of FFA and TG but decreased concentrations of TC and UC and all phospholipid fractions except PE (Table I) After adequate vitamin B_{12} treatment FFA remained virtually unchanged but the TG concentration decreased It was however still higher than in normals The TC concentration normalized after treatment and so possibly did UC The different phospholipid concentrations were all restored to normal after treatment In summary, there were persistently high levels of FFA and TG after treatment but otherwise *fundamentally* normal values

Table II *Changes in lipid and phospholipid concentrations (µmol/l during incubation of plasma (6 hours 37°C)*

Significance of changes within groups tested by paired *t* tests

	FFA	TG	TC	UC	PL	LL	SP	PC	PE
1 *Normal subjects*									
Mean	+150**	−93**	−13	−391**	−19*	+222**	−3	−239**	0
S D	335	25	881	54	45	17	14	31	6
n	20	27	27	17	27	27	27	27	27
2 *PA in relapse*									
Mean	+265**	−113 *	−36	−215**	−23	+139 *	+3	−168	−3
S D	145	121	174	192	87	50	36	92	13
n	31	34	34	30	34	34	34	34	34
3 *PA in remission*									
Mean	+255***	−86**	+54	−233***	+1	+206*	+16	−219***	0
S D	126	84	293	142	73	67	44	94	13
n	15	15	15	14	15	15	15	15	15
Mean difference									
2–1	+155**	−20	−23	+176 *	−4	−83**	0	+71**	−3
3–2	−33	*+53*	+93	*+18*	*+26*	*+68****	*+6*	−52	+3
3–1	+105	+7	+67	+158***	+19	−16	+19	+19	0

Tested by *t* tests for paired (3–2) or unpaired (2–1 and 3–1) groups
Statistical symbols as in Table I

After incubation all groups showed a decrease in UC and a simultaneously diminished PC concentration There was also a parallel increase in LL The concentration of FFA increased and the TG decreased in all groups The PA patients in relapse showed a larger FFA increase and smaller UC and PC decreases than the normals on incubation the increase in LL was also hampered After B_{12} treatment the LL formation on incubation was normalized whereas the expected decrease in UC was still significantly lower than in the normals (Table II)

Incubation of postheparin plasma from the 17 cases in relapse resulted in a very pronounced increase in FFA and a corresponding decrease in TG The decreases in PC and PE and the increase in LL formation were also steeper than in preheparin plasma There was no further decrease in UC compared with the changes in the preheparin samples (Table III) Incubation of postheparin plasma after vitamin B_{12} therapy did not result in any major changes except for significantly steeper increases in PL and LL (Table III)

During the latest period of this investigation 6 cases of PA before and 4 cases also after specific treatment were examined for determination of the initial level of lecithin cholesterol acyl transfer rate All 6 patients showed values within the normal reference range of our laboratory (56–130 µmol/l/h) but after treatment a considerable increase occurred in 3 out of 4 cases Both before and after therapy the rate of lecithin cholesterol acyl transfer was within the normal range related to plasma lipid concentrations (12) The HDL lipid concentration before treatment was also followed in 6 cases in relapse and the concentration of TC in this lipoprotein fraction was significantly lower than that found in a reference group (12)

DISCUSSION

Lipid determinations in PA patients in relapse showed lower concentrations of TC UC and PL but higher concentrations of TG and FFA compared with normal subjects Taking into account TC and PL these findings are in agreement with earlier reports (8) The figures correspond to a lower than normal net esterification of cholesterol and a net decrease in LL formation in PA cases in relapse During 6 hours incubation of plasma the LCAT reaction will be highly dependent on the concentration of the substrates UC and PC The low values of UC and PC can thus explain the low LL formation rate and likewise the low degree of

Table III *Lipid and phospholipid changes (µmol/l) during incubation of plasma (6 hours 37°C) drawn before and 10 min after heparin administration from patients with pernicious anemia (PA) before and after B_{12} treatment*

Significance of paired differences tested by paired *t* tests

		FFA	TG	TC	UC	PL
Before B_{12}	Mean	+288	−131	−39	−169	+1
A Before heparin	S D	167	99	299	114	52
	n	13	17	17	13	17
B After heparin	Mean	+2 540	−564	−106	−184	−55
	S D	964	231	298	109	62
	n	13	17	17	13	17
Mean difference B–A		+2 252***	−433***	−67	− 15	−56***
After B_{12}	Mean	+233	−74	+5	−254	−26
C Before heparin	S D	83	60	256	199	84
	n	5	6	6	5	6
D After heparin	Mean	+2 694	−628	−272	−212	−2
	S D	1 205	129	259	171	31
	n	5	6	6	5	6
Mean difference D–C		+2 528**	−554**	−277	+42	+24
Mean difference D–B		−619	+121	−44	−21	+58**

Statistical symbols as in Table I

cholesterol esterification These findings may also explain the results reported by others (8) indicating a decreased LCAT activity at lower concentration of plasma substrates

In PA cases substrate concentration and changes caused by incubation are lower concerning LCAT but higher concerning lipase activity than in normals Looking at the corresponding changes after B_{12} therapy the substrate compounds PC and UC increase to normal levels and net LL formation is normalized This indicates an increased cholesterol esterification in agreement with the findings by others (8)

The crucial point is however whether the LCAT activity which seems to be lower than normal in absolute figures in cases of PA might be normal in relation to the lipid substrate composition of patients with B_{12} deficiency Both data presented earlier (8) and the determination of lecithin cholesterol acyl transfer rate in the present study speak in favor of this situation As also pointed out (8) UC and esterified cholesterol increased in parallel with a simultaneous increase in LCAT activity after specific treatment

Incubation of postheparin plasma demonstrated the presence of a lipase and phospholipase activity in PA patients The net esterification of cholesterol was unchanged in postheparin plasma In spite of the greatly increased FFA concentration after incubation no further UC decrease could be found in this situation which speaks against acylation as a likely mechanism for the cholesterol esterification in plasma After B_{12} therapy incubation of postheparin plasma showed an additional increase in LL concentration which is probably explained by the higher substrate concentration of PC

Going back to the original observation (1 2 3) of the non stabilization of the erythrocytes suspended in plasma of PA patients after incubation this seems to be explained by the findings in this report as well as in others (8) showing a low LL formation due to the insufficient substrate availability for LCAT and phospholipase As already shown by other authors (8) B_{12} treatment resulted in an increased substrate concentration and cholesterol esterification This finding was confirmed in principle by the present study in an—admittedly—small number of PA patients in whom determinations of lecithin cholesterol acyl transfer rate were performed before and after B_{12} therapy

The elevated concentrations of FFA and TG (Table I) are rather unexpected The relatively low LCAT rate speaks against increased production as a cause of the elevated TG concentration (12) According to our data and those of others (8) a primary deficiency of lipolytic plasma enzymes is evi

	SP	PC	PE
0	+1	−126	−5
9	40	60	12
7	17	17	17
3	−14	−209	−10
4	50	67	17
7	17	17	17
3**	−15	−83***	− 5
4	+8	−207	−2
1	65	105	11
6	6	6	6
2	−18	−223	−15
4	61	94	25
6	6	6	6
8	−26	−16	−13
7 *	+12	−42	0

dently not present in PA cases Possibly a combination of increased lipolysis and decreased degradation of VLD lipoprotein TG in adipose tissue or liver occurs in these cases One explanation for the lowered levels of TC and PL might be a slight malabsorption in PA cases in relapse

As previously shown by our group (4) a prerequisite for a normal erythrocyte suspension stability after incubation is that the LL concentration has reached a certain level (4 5 μg phospholipid phosphorus/ml in the LL fraction of the thin layer chromatogram) If this level is not reached due to an abnormally low LL concentration and/or formation the reduction of ESR after incubation will fail to appear

The small increase in LL does not seem to be caused by LCAT or lecithinase deficiency but rather by a deficiency of substrate lipids in plasma of PA patients The ultimate cause of the changed lipid metabolism in PA patients is still obscure as neither LCAT nor postheparin lipases seem to be primarily deficient Perhaps the low concentration of B_{12} has an influence on the lipid metabolism on the cellular level in intestine liver or adipose tissue

ACKNOWLEDGEMENT

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Determination of ^{58}Co-Vitamin B_{12} Absorption in Pernicious Anemia by Use of Whole-body Counting Reproducibility and Control of Gut Transit Time

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ABSTRACT In 15 patients with pernicious anemia the fractional retention of ^{58}Co-cyanocobalamin (FRB_{12}) without administration of intrinsic factor (IF) averaged 4 9% (range 0–12 7) With administration of IF, FRB_{12} averaged 32 7% (range 26 6–42 3) in the patients and 65 6% (range 38 8–84 6) in 16 control subjects The gut transit time for vitamin B_{12} was evaluated from the excretion of radiopaque pellets given concomitantly with the ^{58}Co-vitamin B_{12} The retention of the pellets was positively correlated to that of the non absorbed ^{58}Co-B_{12} Control of the gut transit time is recommended at each examination of FRB_{12} in order to avoid falsely high values due to the retention of non absorbed ^{58}Co-B_{12} We found a good reproducibility of FRB_{12} when determined in fasting subjects, and it is therefore unnecessary to give the patients a B_{12}-free meal prior to the examination As the FRB_{12} in all probability is only a little lower than the fractional absorption, the present method is applicable for the determination of B_{12} absorption

With today s highly sensitive whole body counters the retention of small amounts of radionuclides can be followed over a long period In this way it has been possible to evaluate the absorption (3 5 6 9 10 11 12 18 19 20 22 23) and the catabolic rate (1 2 14 15) of vitamin B_{12} (B_{12}) from direct measurements

Since only a few data (10 11) have been published on the precision of B_{12} absorption measurements with whole body counting we have examined the reproducibility of the test in normal subjects and in patients with pernicious anemia Furthermore the importance of controlling the gut transit time was examined

MATERIAL AND METHODS

The material comprises 17 patients 7 men and 10 women aged 50–85 years (mean 69) with pernicious anemia in remission for 6 months–12 years (mean 4 years) The matched control material consists of 23 subjects 11 men and 12 women aged 44–98 years (mean 77)

In the examination period neither the controls nor the patients were given parenteral B_{12} The patient material was composed in such a way by age and sex that it was representative of the distribution of the disease in the population (4) Neither materials included subjects with gastrointestinal diseases disorders of the thyroid gland liver diseases leukemia or renal diseases

Radiopharmaceutical

Patients and controls fasted for 12 hours before and 4 hours after administration of ^{58}Co-B_{12} The tracer was given orally as a preparation containing 0 5 μg cyanocobalamin and 0 2–0 4 μCi ^{58}Co-B_{12} dissolved in 30 ml of deionized water Immediately after the subjects were given 25 radiopaque pellets (see below) together with 80 ml of deionized water Intrinsic factor (IF) was given as hog IF in a dose of 100 mg

Whole-body counting

^{58}Co was measured within the 0.200–0 900 MeV energy spectrum with a sensitive well shielded whole-body counter (8) The coefficient of variation for each measurement of the whole-body radioactivity was less than 1%

Estimation of gut transit time

Concomitantly with the administration of ^{58}Co-B_{12} the subjects were given 25 radiopaque pellets (Portex Radiopaque Polyethylene tubing pp 290) weighing 21 mg each (16)

If the X ray of the abdomen taken at day 7 showed one or more pellets the subject was not included in the analysis of the fractional retention of ^{58}Co-B_{12} (FRB_{12}) the retained pellets being taken to suggest the presence of non absorbed ^{58}Co-B_{12} in the intestines In the following

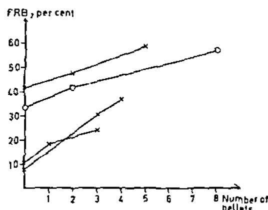


Fig 1 The relationship between the fractional retention of *⁵⁸Co-vitamin B₁₂ (FRB₁₂) and the number of radiopaque* polyethylene pellets retained in two patients with pernicious anemia In one patient (O) the retention was examined following administration of intrinsic factor (IF) in the other (×) three examinations were made one with administration of IF and two without Each graph is drawn *from three observations 7 10 and 14 days after oral ad*ministration of ⁵⁸Co-vitamin B_{12} the results from day 7 being given furthest to the right

the term normal gut transit time means that all pellets had been excreted before the measurement at day 7

In order to find out whether the passage of the pellets reflected that of ⁵⁸Co-B_{12} the following examinations were carried out 1) In 2 patients with 3–8 pellets retained on day 7 the ⁵⁸Co-B_{12} retention was followed until the pellets been excreted 2) In 11 subjects with a normal gut time the retention was measured 7 and 14 days the oral administration of ⁵⁸Co-B_{12}

Fractional retention of ⁵⁸Co vitamin B_{12} (FRB₁₂)

Each determination of the retention of ⁵⁸Co B_{12} requires three measurements with the whole body counter namely of the background activity and of the activity 2 hours and *T* days respectively after the administration of ⁵⁸Co B_{12} The retention fraction was calculated from the formula $N_{T\,days}/N_{2\,hours}$ where $N_{T\,days}$ and $N_{2\,hours}$ are the number of counts *T* days and 2 hours respectively after the administration

Schilling test

The Schilling test had been performed in 10 of the patients as described by Schwartz et al (21)

Procedure

The investigation was carried out as follows 1) The reproducibility of the FRB_{12} determination was evaluated from two consecutive examinations in 10 controls and 15 patients not given IF The reproducibility when IF was administered was determined in 13 of these patients When the subjects with a slow gut transit time had been excluded the three groups numbered 7 controls and respectively 12 and 12 patients 2) *A single determination* of FRB_{12} was made in 23 controls and 17 patients not given IF Seven controls and 2 patients were excluded owing to a slow gut transit time A single determination of FRB_{12} when IF had been given was made in 15 of the patients Two of them were excluded for the above reason

RESULTS

Fig 1 shows the relationship between the retention of B_{12} and of the pellets in 2 patients with unexcreted pellets on day 7 FRB_{12} is clearly positively correlated to the number of pellets retained

Table I gives the FRB_{12} determined on days 7 and 14 A slight fall in FRB_{12} is seen in 9 of the 11 subjects but the difference is not significant (Wilcoxon rank sum test for paired data $0\ 02<p<0\ 05$)

The determination of the reproducibility of FRB_{12} in the controls showed a standard deviation (S D) of 6% for a mean value of about 60% corresponding to a coefficient of variation (CV) of 10% In the patients not receiving IF S D was 3% and CV 60% the corresponding figures being 5% and 17% in those receiving IF

Table II gives the value for FRB_{12} in the total material with and without administration of IF FRB_{12} averaged 65 6% in the controls, 4 9% in the patients not given IF and 32 7% when IF was given

Table III compares the results from the Schilling test and the determination of FRB_{12} with and without administration of IF in 10 patients There was a highly significant correlation between the results

Table I *Fractional retention of ⁵⁸Co-vitamin B_{12} (FRB_{12}) in four patients with pernicious anemia and in seven controls 7 and 14 days after a single oral tracer dose*

FRB_{12} (%) Day 7	Day 14
Patients	
5 8	4 6
5 9	4 5
4 6	0 4
0 0	0 0
Controls	
51 2	50 7
60 6	58 9
75 9	56 2
38 8	40 3
51 8	49 4
43 0	42 9
67 1	65 6

from the two tests ($r=0\ 86$ $N=20$ $p<0\ 001$) It should be noted that the mean value for the excretion percentage found at the Schilling test is about 60% of the corresponding FRB_{12} value in patients not given IF as against 40% in patients given IF

DISCUSSION

Gut transit time

Hinton et al (16) found a good concordance between the gut transit time evaluated by means of pellets and of small particled substances such as carmine and ^{51}Cr labelled sodium chromate Our results confirm that radiopaque pellets are a good marker of the gut transit time of ^{58}Co-B_{12} The excretion of the pellets thus parallelled that of the non absorbed ^{58}Co-B_{12} (Fig 1) and when all the pellets had been excreted so had all the non absorbed ^{58}Co B_{12} (Table I) The practical consequence of this is that the gut transit time should be controlled at each examination of FRB_{12} as this will eliminate the main source of falsely high values that is the retention of non absorbed ^{58}Co-B_{12} on the last day of the examination Provided this is done the examination can generally be ended on day 7 instead of day 14 as recommended by for instance Adams et al (3)

Fractional retention of B_{12}

Adams and Boddy (1) showed that following parenteral administration of a tracer dose the excretion of ^{58}Co-B_{12} is 0 4–1 3%/day during the first 5–10 days As the peak concentration of radioactive B_{12} in plasma after the administration of an oral tracer dose is obtained after 8–12 hours (7) it seems reasonable to assume that the excretion following oral administration is of a similar magnitude The FRB_{12} measured at day 7 is thus in all probability only a little lower than the fractional *absorption* which means that the method is applicable for the determination of B_1 absorption

Table II *Fractional retention of ^{58}Co-vitamin B_{12} (FRB_{12}) in controls and patients with pernicious anemia with and without administration of intrinsic factor (IF)*

	N	FRB_{12} (%)	
		Mean	Range
Controls	16	65 6	38 8–84 6
Patients			
Without IF	15	4 9	0–12 7
With IF	13	32 7	26 6–42 3

Table III *Schilling test and fractional retention of ^{58}Co vitamin B_{12} (FRB_{12}) without and with administration of intrinsic factor (IF) in ten patients with pernicious anemia*

	Excretion (%)	
	Mean	Range
Schilling test		
Without IF	3 1	0 4–7 9
With IF	13 2	8 6–19 9
FRB_{12}		
Without IF	5 1	1 3–11 9
With IF	32 0	26 6–42 3

Reproducibility of the FRB_{12} determination

Unlike Finlayson et al (10 11) we found a good reproducibility of FRB_{12} when determined in fasting subjects Finlayson et al concluded that the absorption of B_{12} varies highly in the individual fasting subject Furthermore that the patient should be given a B_{12} free meal prior to an examination of the B_{12} absorption (11) Our results accordingly do not support the latter conclusion

Determination of FRB_{12}

Our results from single determinations of FRB_{12} show that the value differed greatly between the patients given IF and those not given IF as well as between controls and patients not given IF (Table II) Table IV lists the reported results Only two other studies (20 23) deal with FRB_{12} determined with and without administration of IF in patients with pernicious anemia and in both the results overlap which makes a comparison somewhat pointless In the study by Tappin et al (23) the overlapping might be due to the lack of a control of gut transit time

FRB_{12} and Schilling test

The comparison between the results from the Schilling test and the whole body counting (Table III) suggests that the Schilling test results in a relatively higher excretion percentage the smaller the absorption percentage Compared with whole body counting the results will consequently tend to differ less

Table IV *Reported results on the fractional retention of ⁵⁷Co vitamin B_{12} (FRB_{12}) in controls and patients with pernicious anemia*

		Controls			Patients with pernicious anemia					
					Without IF			With IF		
	Day of 2nd measurement		FRB_{12} (%)			FRB_{12} (%)			FRB_{12} (%)	
Authors		*N*	Mean	Range	*N*	Mean	Range	*N*	Mean	Range
Reizenstein et al (20)	7	10	61	38–81	3	3	0–8 8	3	15 3	4–31
Tappin et al (23)	4–7	27		21–78	11		2 7–34 8	7		16 3–45 3
Irvine et al (18)	7	10		37–98	12		1–23			
Simpson & Shearman (22)	7	27		>20	5		<10			
Irvine et al (19)	7	35	64 3	24–98	27	9 9	0–25			
Cottral et al (9)	9	11	46	30–66	5	10	3–16			
Adams et al (3)	14	26	45 7	19 1–73 2	26	6.3	0 3–16 2			
Present investigation	7	*16*	*65 6*	*38 8–84 6*	*15*	*4 9*	*0–12 7*	*13*	*32 7*	*26 6–42 3*

between patients given IF and those not given IF It is therefore possible that for a distinction between patients with pernicious anemia and patients with B_{12} malabsorption whole body counting is more applicable than the Schilling test

CONCLUSION

It can be concluded that FRB_{12} determined by whole-body counting gives the best approximation obtainable to the absorption of B_{12} It is a prerequisite that the gut transit time is controlled at each examination in order to avoid falsely high values The method is applicable to the diagnosis of pernicious anemia Furthermore it is applicable as a reference test for other methods for instance determination of the fractional B_{12} absorption by means of incomplete stool collection (17)

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An Eleven-year Follow-up on 64 Subjects with M-components

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ABSTRACT In a medical health survey in 1964 of 6995 subjects, 64 persons with M-components were detected by serum electrophoresis One had clear cut signs of myelomatosis and one had lymphatic leukemia Fleven years later 27 persons had died In the case with lymphatic leukemia the disease had changed after 6 years into a myelomatosis One person died after 9 years from a malignant lymphoma Among the 37 persons still alive there seemed to be no clustering of diseases The whole series represents an observation time of 487 years No more myelomas or lymphomas were detected One M component had disappeared

In a survey of 6995 persons in a district of Sweden (1) M-components of Ig were found in 64 Most of the M-components detected 41 were trace components with a concentration of 5 g/l or less Two follow up studies have been published (3 4) A new study was carried out in 1975 exactly 11 years after the initial screening and the results are presented below

MATERIAL AND METHODS

Thirty-seven persons were still alive and it was possible to get some information on all The same nurse as in 1964 and 1969 visited 31 of these persons interviewed them and drew the blood samples Medical records X rays and biopsy specimens were placed at my disposal by many hospitals

Plasma samples from 31 persons were sent to the Laboratory of Clinical Chemistry Malmo General Hospital for examination The previous method with paper electrophoresis is no longer in use The new method described by A Grubb (5) may give a difference within the 20% level in the quantitation of M components It was not possible to examine old and fresh samples at the same time with the new technique Thus one cannot make a really reliable absolute comparison between concentrations in 1964 and 1975

RESULTS

The analysis of M-components gave the following results Differences (g/l) of ±5 +6–10 +11–15 +16–20 in 23 6 1 1 persons respectively Significant increases of 16 and 14 g/l were noted in two cases Nothing in the clinical records indicated disease in these subjects In one case the M component had disappeared and been substituted by an oligoclonal gammapathy (case C 2 in ref 2) Owing to the methodological difficulties it was impossible to draw any conclusions regarding small differences in the trace components which constitute the main part of the material but they are obviously of no real importance No sample had become normal

The state of health of the 31 persons who were personally interviewed was fairly good for their age No information was available on contracted blood or skeletal disease in the other six living persons

Since 1964 27 persons had died 16 of whom are reviewed in a previous paper (4) Table I is the extended list with data on all 27 In very few cases had autopsy with examination of the skeleton been performed but there is no indication of death from active myeloma Five cases seem to have special importance and their case records are given below

CASE REPORTS

Case 6350

Male born in 1877 When examined in 1964 he was free from symptoms There was no enlargement of lymph nodes liver or spleen WBC 20 500 53% lymphocytes The bone marrow was rich in lymphatic cells and there was no increase in plasma cells The M-component IgGK was 18 g/l (22 g/l in 1969) Hospitalized in 1970 due to back pains and bronchopneumonia He lost weight and became bedridden The M-component was 60 g/l The

Table I *Data concerning 27 subjects who have died since 1964*

Subj no	Time of death (mo /y)	Age at death (y)	Cause of death
6497	9/64	75	Malignant tumour
7953	10/64	61	Complication of cholecystectomy
3396	2/65	84	Senility
5457	10/65	70	Pulmonary embolism after appendectomy
3561	6/66	71	Cerebrovascular lesion
7006	11/66	69	Myocardial infarction
8250	12/66	84	Myocardial and cerebral infarction
4432	12/66	70	Myocardial infarction
8469	1/67	70	Cerebrovascular lesion
3860	3/67	70	Pancreatic cancer
5062	7/67	66	Cerebrovascular lesion
6230	10/67	87	Prostatic cancer
4070	11/67	82	Cardiosclerosis
6844	1/68	77	Myelomatosis bronchopneumonia
6794	5/68	78	Cardiosclerosis
7913	6/69	67	Malignant tumour
8420	1/70	81	Cardiosclerosis
6350	1/71	93	Myelomatosis
7082	6/72	87	Myocardial infarction
6140	6/73	69	Malignant lymphoma
8416	7/73	65	Myocardial infarction
5943	1/74	72	Cerebrovascular lesion
8864	3/74	74	Trombocytopenia cerebellar hemorrhage
1354	5/74	82	Megakaryocytic myelosis
2500	11/74	90	Senility
6946	4/75	67	Myocardial infarction
4879	6/75	84	Diverticulitis?

bone marrow contained 35% plasma cells No signs of kemia in the blood X ray vertebral compressions He in Jan 1971 This case represents a transition from a nphatic leukemia to myelomatosis with a simultaneous steep rise of the M-component

Case 6140

Male born in 1903 In 1964 the M-component IgGL was 13 g/l His main complaint was that he bruised easily Coagulation studies were normal In 1969 his M component was 20 g/l In 1972 when he fell ill with a septicemia caused by E coli swollen glands were observed in the inguina and axillae The bone marrow as well as the peripheral blood seemed to be normal X ray of the skeleton did not disclose any destructions The M-component had risen to 40 g/l and light chains were excreted in the urine Treatment with melphalan had no effect and his condition deteriorated He was admitted to hospital for the last time in 1973 and succumbed to septicemia in June Autopsy not permitted

Rausing (6) has reexamined a lymph node biopsy from 1972 electron microscopically and established a safe diagnosis of malignant lymphoma centrocytic/centroblastic small cleaved sclerotic

Case 8864

Female born in 1899 Previously described as case B2 in a family study (2) Her brother who had an M-component too died at the age of 80 after a stroke She was hypertensive for many years and had been operated upon for an ovarian cyst in 1972 In 1964 her M-component IgAL was 5 g/l It did not change much in the first five years

In June 1973 she sought medical advice for a hematoma of the left upper arm Hb 99 g/l WBC 5400 thrombocytes 114 000 During the next few months she experienced several episodes with small hematomas She was admitted to hospital in Jan 1974 No pathological lymph nodes were found the liver and spleen were not enlarged X ray of the skeleton was normal Hb 83 g/l WBC 4 500 thrombocytes 20 000 The bone marrow picture was not consistent with leukemia or myeloma Haptoglobin 0 3 g/l (low) and reticulocytes 7 5–12 5% (high) The M-component was 17 g/l no light chains in the urine The cause of hemolysis and thrombocytopenia was unknown A few immature cells and nucleated red cells were sometimes detected in the peripheral blood but a diagnosis of leukemia could not be made She died suddenly in her home in March 1974 In accordance with Swedish law a standardized police autopsy had to be performed The cause of death was a cerebellar hemorrhage Histologic examination of the bone marrow and spleen was not performed

Case 1354

Male born in 1892 His IgG M-component rose from 8 g/l in 1964 to 13 g/l in 1973 In this year he had a progressive heart failure He was now anemic Hb 80 g/l WBC 8 000 Thrombocytes 95 000 The bone marrow showed no signs of myeloma A few immature cells were sometimes pres

ent in the peripheral blood Several biopsies of bone and spleen did not permit a safe diagnosis although a myeloproliferative disease was suspected He died in May 1974 At autopsy the vertebral bone marrow was very rich in cells the main constituent being megakaryocytes The final diagnosis was acute megakaryocytic myelosis

Case 4879

Female born in 1891 The study in 1964 had revealed an IgA M-component of 2 g/l She refused to take part in the follow up examinations The available hospital records contain the following history In 1974 breast cancer operation and radiotherapy In 1973 abdominal pains gall stones at X ray In 1974 a barium enema disclosed a narrowing of the sigmoid in an area of 10 cm with many diverticles After an episode of ileus she needed a transversostomia She was in bad shape and major surgery could not be done There was no biopsy from the sigmoid area X ray of the skeleton disclosed osteopenia and a *compression of the 12th thoracic vertebra She died in* June 1975 no autopsy

This case is poorly examined and a safe diagnosis can not be made It was assumed that she had a perisigmoiditis but a neoplastic disease cannot be ruled out

COMMENTS

In this follow up no cases have been completely lost but full information is lacking in six In the initial survey one case of myelomatosis was detected The diagnosis was suspected in two other cases but was not proved at the time of their death from other diseases (cases 8420 and 7006) One old man (case 6350) with a silent lymphatic leukemia from the beginning had an increase in the M component after 5 years At his death it became evident that he had had a clearcut transmutation from leukemia to myeloma The total series thus contains 2 cases of myelomatosis One patient (case 6140) was initially suspected of having a serious disease but no diagnosis could be made After 8 years he had an overt disease with malaise and enlarged lymph nodes His M component grew fairly large 40 g/l and he excreted light chains in the urine The diagnosis was without doubt malignant lymphoma One woman died after 10 years from a pancytopenia Her M-component had increased There were no signs of myeloma or acute leukemia and the connection may be fortuitous The same may be said about an old man who died after 10 years from acute megakaryocytic myelosis

We should perhaps have an open mind on these things and continue to register unexpected and unusual final diseases in people with seemingly benign gammapathies

In conclusion it may be said that this survey represents an observation time of 487 years and that malignant disease of immunocytes connected with an M-component has occurred only thrice two myelomas and one lymphoma In none of the others has a trace component increased paralleling progression of disease *At a time when interest in* electrophoretic screening is growing and ever more M-components are detected it is reassuring that even in the long run the majority represent a benign condition

ACKNOWLEDGEMENT

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Different Composition of the Eosinophilic Bone Marrow Pool in Reactive Eosinophilia and Eosinophilic Leukaemia

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ABSTRACT The composition of the eosinophilic cell series in the bone marrow has been analysed in 10 patients with a pronounced reactive eosinophilia (RE) and in 2 with eosinophilic leukaemia (EL) An impaired differentiation of the eosinophils was found in the EL patients compared with the RE group Thus the ratio of eosinophilic promyelocytes+myelocytes segmented eosinophils was 9 2 and 9 1, respectively, in the patients with EL and 0 1-3 1 (average 1 3) in the RE patients It is suggested that EL is characterized by an impaired differentiation of the eosinophilic bone marrow cells and that the recognition of this abnormality is of value in the differential diagnosis between EL and RE

The differentiation of reactive eosinophilia (RE) from the rare neoplastic condition eosinophilic leukaemia (EL) is sometimes difficult since comparably increased numbers of eosinophilic leukocytes may be present in peripheral blood and bone marrow in the two conditions The existence of EL has even been questioned and it has been suggested that the majority of cases presented as EL have in fact been leukaemoid reactions (1 2 5) The demonstration of chromosome abnormalities in bone marrow preparations dominated by eosinophilic cells however supports the existence of haematologic malignancies affecting the eosinophilic cell series (3 4 7 9 11 14 15 17) Karyotypic analyses have also demonstrated that several cases of EL can be distinguished from classical chronic myeloid leukaemia with prominent eosinophilia (1 3 7 9 16 17 19)

Although chromosome studies of bone marrow cells in cases with a severe infiltration of eosinophils are important in the differentiation of RE from EL karyotypic studies demand special proficiencies and are time consuming Furthermore it is possible that some cases of EL lack chromosomal abnormalities (1 6 13) A simple and rapid method which with reasonable certainty differentiates RE from EL would therefore be of value In the present work it is demonstrated that a morphologic analysis of the composition of the eosinophilic cell series in the bone marrow may give information about the nature of the eosinophilic bone marrow infiltration

MATERIAL AND METHODS

Patients with RE

Ten patients with pronounced eosinophilia and a considerable increase in eosinophilic cells in the bone marrow were selected (patients 1-10 Table I) In no case did the clinical course warrant a diagnosis of leukaemia The percentage of eosinophilic cells in the bone marrow was 15-44 (mean 25.5)

Patients with EL

Two patients with severe infiltration of eosinophilic leukocytes in the bone marrow and with an unequivocal haematologic malignancy were studied (Table I) One of them a 40-year-old male (patient 11) had anaemia 57 g/l and leukocytosis with WBC 47.5×10^9/l The differential count showed 88% eosinophilic cells No blast cells were found In the bone marrow 58% of the cells were eosinophils Repeated chromosome analyses revealed a conspicuous marker chromosome in 100% of the bone marrow metaphases described in detail elsewhere (17) There was no Ph^1-chromosome He had splenomegaly and fine needle aspiration biopsy demonstrated myeloid metaplasia with a predominance of eosinophilic cells

The other patient (no 12) a 26-year-old male was first seen in Dec 1974 because of leukocytosis He had been well until one month previously when he saw his doctor

Table I *Clinical data on the patients*

Pat no	Sex	Age (y)	Diagnosis or symptoms	No of eosinophils/l blood × 10^{9a}	Comments
1	♀	20	Acute abdominal pain fever ascites	21 8	Spontaneous and rapid regression of all symptoms Followed for 1 year without relapse of symptoms or eosinophilia
2	♂	21	Hodgkin s disease	19 6	
3	♀	73	Bronchial asthma	2 0	
4	♀	50	Polyarteritis nodosa	7 5	
5	♂	49	Dermatitis herpetiformis	7 6	
6	♂	50	Hodgkin s disease	1 1	
7	♂	23	Wilson s disease	0 6	Probably allergic reaction due to penicillamine
8	♀	44	Trichinosis	3 0	
9	♀	77	Bronchial asthma	4 6	
10	♂	61	Cholecystitis	6 2	Regression of eosinophilia after cholecystectomy
11	♂	50	Eosinophilic leukaemia	42 0	Isochromosome 17 in all bone marrow metaphases
12	♂	26	Eosinophilic leukaemia	46 1	Extra C group chromosome in peripheral blood cells

[a] Estimated from WBC and differential count

because of ache in the shoulder region and he reported *having had some bruises without apparent trauma some* weeks earlier A few days later his temperature rose to 38 C He had no symptoms of upper respiratory infec tion There was no history of exposure to infectious disease nor of allergy or parasitic disease

Physical examination revealed no abnormal findings and no rash or petechiae were seen in the skin or surfaces Apart from that the spleen was just below the left costal margin No lymph nodes were Urinalysis was negative, Hb 130 g/l platelets 60× 10^9/l The WBC was 98× 10^9/l with 3% promyelocytes 4% myelocytes 24% neutrophils 47% eosinophils 12% basophils 6% lymphocytes and 1% monocytes The NAP score was low and the serum B_{12} level elevated (5 148 pg/ml) A bone marrow aspiration yielded a very cellular marrow with predominantly neutrophilic and eosinophilic cells Chromosome analyses were performed on unstimulated and PHA stimulated cultures from peri pheral blood In the unstimulated culture only 2 meta phases were found but both displayed clear abnormali ties including an extra C group chromosome This aberra tion was also present in about half of the metaphases analysed from the PHA stimulated culture The remain *ing cells in this culture had normal chromosomes* No Ph^1-chromosome was found

The patient was treated with busulphan 6 mg a day and within a month the WBC decreased to 10× 10^9/l with 33% eosinophils *He was kept on 2 mg busulphan a day* for the next month but the WBC rose to 98× 10^9/l with 33% eosinophils The busulphan dose was again in creased and within a fortnight WBC decreased to 10× 10^9/l with 36% eosinophils

At the same time the patient s condition changed rapidly and the leukaemia underwent a metamorphosis He developed multiple subcutaneous tumour masses and *also an intraorbital tumour which caused diplopia* The tumours consisted mainly of myeloblasts but some also showed an increase of eosinophils The tumours were radiosensitive and some of them disappeared after local X ray treatment *The* patient s condition *however* deteriorated and he died two months later Autopsy revealed haemorrhagic diathesis and massive leukaemic infiltrations in the enlarged spleen liver kidneys and the meninges

Morphologic studies of the bone marrow eosinophils

Bone marrow smears were stained with May-Grunwald Giemsa Five hundred eosinophilic cells were examined and the percentages of eosinophilic cells at different stages *of maturation were determined* Since eosinophilic gran ules sometimes obscured the nuclear structure making the distinction between promyelocytes and myelocytes uncertain these cells were pooled into one group The examination was performed *by one technician* especially trained in haematologic cytology without knowing the diagnoses of the patients The morphologic criteria given by Heilmeyer and Bergmann (12) were used for classi fication of the cells

RESULTS

The results of the differential counts of the bone marrow eosinophils are given in Table II A more pronounced maturation within the eosinophilic cell series was found in the RE patients compared with those with EL Thus the ratio of eosinophilic pro

Table II *Differential counts of eosinophilic bone marrow cells (%) and the ratio of eosinophilic promyelocytes+myelocytes segmented eosinophils*

Pat no	Eosinophilic promyelocytes+ myelocytes	Eosinophilic metamyelocytes	Non segmented eosinophils	Segmented eosinophils	Eosinophilic promyelocytes+myelocytes segmented eosinophils
RE patients					
1	21 8	17 0	25 0	36 2	0 6
2	44 8	18 0	18 0	19 2	2 3
3	26 4	19 8	21 4	32 4	0 8
4	3 6	3 4	31 8	61 2	0 1
5	46 8	14 2	21 8	17 2	2 7
6	34 4	8 4	22 0	35 2	1 0
7	53 4	14 0	15 6	17 0	3 1
8	34 3	13 2	19 2	33 3	1 0
9	28 6	11 2	22 2	38 0	0 8
10	24 2	11 6	28 0	36 2	0 7
Mean	31 8	13 1	22 5	32 6	1 3
EL patients					
11	69 8	3 6	19 0	7 6	9 2
12	50 2	28 5	15 8	5 5	9 1

myelocytes+myelocytes segmented eosinophils was 0 1–3 1 (average 1 3) in the RE patients and 9 2 and 9 1 respectively in the two patients with EL

In the RE patients with the highest eosinophil counts there was no tendency to an impaired maturation of the eosinophilic bone marrow pool (Tables I and II) The defective differentiation of the eosinophilic bone marrow cells in the patients with EL was therefore in all probability not due to their pronounced eosinophilia

DISCUSSION

As stressed by several authors (1 2 5 13 18) the diagnosis of the rare disease EL must be considered very critically before it is accepted Two of our patients had several stigmata advocating the diagnosis of EL. In addition to the pronounced eosinophilia in peripheral blood and bone marrow they had splenomegaly due to leukaemic infiltration and chromosome abnormalities were found in both Although several traits in our patients might suggest that their disease was merely a variant of chronic myeloid leukaemia (CML) the Ph¹-chromosome was not found in any of them and it must therefore be concluded that their malignant blood disorders differed from classical CML

In normal bone marrow an average of 37% of the sparsely occurring eosinophils have been reported to be promyelocytes+myelocytes and 37% are fully mature segmented eosinophils (10) The findings of very similar figures in our patients with RE indicate that RE may be associated with a normal maturation of the eosinophilic bone marrow pool It is probable therefore that the discrepancy in eosinophilic maturation between the RE and EL patients reflects an essential difference between the two conditions It is well known that the capacity of immature bone marrow cells to differentiate towards more mature cell types may be blocked in various types of granulocytic leukaemia (8) It is therefore reasonable to assume that such an abnormality should also characterize leukaemic eosinophilic cells and that the accumulation of immature eosinophils in the bone marrow of the EL patients may be regarded as a sign of haematologic malignancy

The clear-cut difference found here in the composition of the eosinophilic bone marrow pool between the patients with RE and those with EL suggests that a simple morphological examination of the bone marrow with special regard to the eosinophilic cell series may be of value in the differential diagnosis between RE and EL

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Antibodies against Human Cholinergic Receptor Proteins in Patients with Myasthenia Gravis Studies during Immunosuppressive Treatment

PRELIMINARY REPORT

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Myasthenia gravis is a disease with the characteristic symptoms of abnormal fatigability and muscular weakness This is caused by a defective transmission across the neuromuscular junction

In recent years an immunoglobulin capable of binding in vitro to cholinergic receptor preparations has been found in the majority of patients with myasthenia gravis (1 4) The occurrence of this receptor antibody — together with observations during lymph drainage of patients with myasthenia gravis in whom retransfusion of cellfree lymph accentuated the myasthenic symptoms (1)—clearly suggests that antibodies against postsynaptic membrane proteins may interfere with neuromuscular function

METHODS

The amount of IgG binding to a human skeletal muscle receptor preparation was measured by a method similar to that used by Appel (1) and Lindstrom (1) Human skeletal muscle was collected from amputated legs at the time of operation The specimen was homogenized at 0°C in 4 volumes of 0 05 M Tris HCl 0 1 NaCl 0 001 M EDTA pH 7 4 with addition of 10^4 IU Trasylol/l The homogenate was centrifuged at 20 000 g for 20 min at 4°C the pellet washed once and resuspended in 3 volumes of the same buffer containing 1.5% Triton X 100 This mixture was stirred at room temperature for 90 min and then centrifuged at 20 000 g for 20 min at 4°C The binding capacity of the supernatant for ^{125}I-α neurotoxin from Naja naja siamensis was 5–10 nmoles/l The recovery of toxin binding sites was 2–5 pmoles/g muscle (wet wt) The supernatant was stored in aliquots at −80°C until use The cholinergic receptor preparation 10–20 pmoles was incubated for 3 hours at 37°C with 200 pmoles of ^{125}I-α neurotoxin The toxin was labeled to a specific activity of 10 Ci/mmol according to the chloramin T method (2) The labeled toxin–receptor complex was separated from excess toxin by gel filtration on Sephadex G 200 at room temperature Subsequently 0 2–0 5 pmoles of the toxin–receptor complex was incubated with 10 µl of serum or plasma for 16 hours at 4°C Rabbit antihuman IgG 300 µl was added and the tubes were allowed to stand for 3 hours at 37°C The precipitate was separated by centrifugation in a Beckman minifuge (8 000 g 5 min) and was washed once with 200 µl of buffer After alkaline hydrolysis and neutralization the radioactivity and protein content of the precipitate were determined

The amount of receptor antibody (a) compared with the amount of normal IgG (b) in the sample was calculated according to

$$\frac{a}{b} = \frac{\text{cpm for patient sample} - \text{cpm for normal plasma}}{\text{cpm for normal plasma}}$$

The cpm value for the normal plasma was corrected to the same amount of IgG as the patient sample The concentration of receptor antibody (arbitrary units) was then calculated as

total concentration of IgG $\times a/(a+b)$

The normal plasma pool which was the same in all experiments was composed of plasma from 50 apparently healthy blood donors Values above 2 S D for the normal pool were considered abnormal Immunological IgG determinations were made using an automated turbidometric method (3) Protein determinations were carried out according to Lowry et al (5)

Clinical evaluation of the 40 MG patients was made by one of us (G M) without knowledge of the results of the biochemical tests using a scale from 0 to 4 (0=no muscular symptoms at all 1=slight weakening after long period of contractions 2=moderately severe symptoms 3=severe symptoms hospitalization often necessary 4=complete paralysis of vital muscle groups intensive care often necessary) Immunosuppressive treatment (ACTH corticosteroids azathioprine) was given according to procedures reported elsewhere (1)

RESULTS

In 34 (85%) of 40 patients the amount of IgG which binds to receptor–toxin complex was significantly higher than in the normal plasma pool None of 29 normals and pa

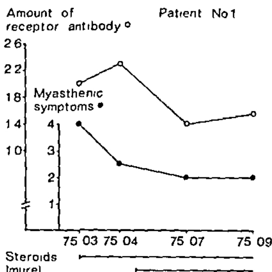


Fig 1 Effect of treatment with azathioprine and steroids on receptor antibody concentration and clinical symptoms

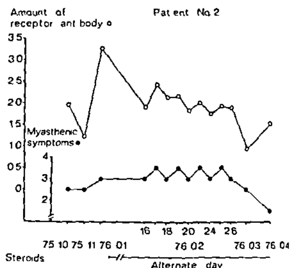


Fig 2 Effect of alternate-day steroid treatment

tients with various other diseases had significant amounts of the antibody There was a definite correlation between the severity of the disease and the amount of receptor antibody the correlation coefficient for the whole patient population was 0 44

No correlation at all was found between the amount of receptor antibody and the titre of antibodies to striated muscle (6) Several patients had no detectable antibody to striated muscle and high amounts of receptor antibodies and one patient had no detectable receptor antibody and a high titre (1 40) of muscle antibodies

In 5 out of 9 patients who were followed during the of immunosuppressive treatment a close covari was found between the amount of receptor antibody and the clinical condition The results of 3 of them are shown in Figs 1–3

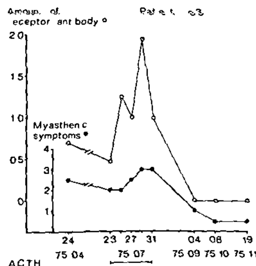


Fig 3 Effect of ACTH treatment

DISCUSSION

Using the method described here it is possible to demonstrate an IgG which binds to cholinergic receptor structures in plasma samples from most myasthenic patients

When the patients are viewed as a group the correlation between the amount of this antibody and the severity of the myasthenic symptoms is rather poor It is much better when the individual patient is followed during immunosuppressive treatment

Of special interest are the rapid variations of receptor antibody concentration during ACTH and steroid treatment These changes in receptor antibody concentration did not follow the variations of total IgG concentration It was shown in a previous paper (4) that the receptor antibody was related to the F(ab $)_2$ fragment of IgG subclass 3 This might partly account for the rapid variations of antibody concentration since IgG 3 has a much shorter biological half life than the other IgG subclasses

The close covariation of clinical symptoms and receptor antibody certainly supports the theory that this autoantibody might be involved in the development of myasthenic symptoms

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Studies on the Pelger Anomaly in Iceland

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ABSTRACT An Icelandic family, containing 15 members with Pelger anomaly, is reported Affected individuals in two branches of the family, living in the south-east and east of Iceland, have been traced to common ancestors born 200 years ago The results of scanning of blood films from approximately 20 % of the population of Iceland suggest that the Pelger family described contains the only mutation of this kind in Icelanders

The distribution of the Pelger anomaly is worldwide and has been detected in Caucasians Orientals and Negroes (14) In the Scandinavian countries it has been reported in Sweden (5 7 8) Denmark (13) Norway (9) and Finland (2)

The first Icelandic family containing 12 members with Pelger anomaly was reported in 1963 (3) and an American Icelandic family in which the American mother and her fourth child had the Pelger anomaly was reported in 1974 (4)

Acquired forms of Pelger like changes in the neutrophil leucocytes are occasionally observed mainly in association with leukaemias (14) An Icelandic family with acquired Pelger like leucocytes and with an abnormal blood marrow clone in two family members has been published (10)

A female patient in a mental hospital born in 1944 was diagnosed with Pelger anomaly in April 1975 She her brother and father who both had the anomaly had ancestors in common with members of the Pelger family reported previously (3) This led to further pedigree studies on the family and a review of the number of Icelanders scanned for this anomaly in 1960–75 The results of these studies are summarized in this report

MATERIAL AND METHODS

The number and types of individuals scanned for the Pelger anomaly are given in Table I Blood films from all individuals investigated were stained by the May-Grunwald Giemsa technique (1) and inspected by the senior author (Ó J)

Pedigree studies

With the aid of two large Icelandic family records (6 11) it has been possible to trace the two branches of this family to common ancestors born 200 years ago (Fig 1) The Pelger family members reported in 1963 are V 10 VI 2–7 and VII.3–21 and those diagnosed in 1975 are VI 1 and VII 1 Both these family branches are traced to two siblings of 13 in generation III nos 11 and 13 The equiprobable ancestral parents born in 1775 are nos 7 and 9 in generation II

The homesteads of the two family branches are shown in Fig 2 They are located in two neighbouring counties on the south-east and east coast of Iceland

Many of the Pelger family members have lived in the same county for many generations but at least five members have moved to the east fjords in the neighbouring county (III 1 3 6 III 11 V 13) Some have moved to other counties in Iceland more distant from their homes and several emigrated to North America in the last century (IV 5 9 V 7 14 15 18) Childhood death and childlessness are manifested in many large sibships of the last century For example 16 members are present in sibship V 5–20 where four sibs had no children (V 6 9 12 19) four died at an early age (V 8 11 16 17) and four emigrated

Genetic analysis of the pedigree data

Twenty-nine individuals were present in 8 sibships excluding those of the two oldest affected parents in the two branches of the family The average sibship size was 3 6 The segregation and sex ratios calculated (Table II) fit the figure expected for an autosomal dominant gene with full penetrance

PELGER · PROBABLE PELGER · NORMAL · UNINVESTIGATED · PROPOSITI (AE)


Fig 1 Pedigree of the Icelandic Pelger family

Results of scanning for the Pelger anomaly

By scanning blood films of individuals recorded in Table I 15 members belonging to the Icelandic family (Fig 1) and two affected members in an American Icelandic family have been detected No additional affected individuals found by scanning 83% of the medical district veyed in 1964 (Table I)

DISCUSSION

As shown in Fig 1 the parents, V 4 and V 10 are second cousins There was a chance that they were both affected heterozygotes The father died in 1946 None of their five affected children had unsegmented neutrophils characteristic of the homozygous form of Pelger anomaly in rabbit and man (5) No loss of foetuses or children due to infection at an early age was known in the family

Of the ambulatory patients scanned and referred by practising family physicians and specialists approximately 90% live in Reykjavík and neighbouring towns and counties About 10% of the individuals surveyed are from more distant places over 50 km from Reykjavík Relatively few inhabitants from the most remote counties in the north and

Table I Icelandic population scanned for the Pelger anomaly in 1960–75

Total population of Iceland 216 000 in Dec 1974

	No of persons investigated	Parent population	Scanned persons (%)
Ambulatory patients			
In Reykjavík	30 000	80 000	37 5
Outside Reykjavík	10 000	130 000	7 7
Inhabitants in Au Sk county (1969)	1 200	1 441	83 3
Total	41 200	211 441	19 5

Table II *Segregation and sex ratio*

	N	Ratio (%)
Propositi	2	
Affected parents	2	
Affected siblings	11	40 7
Unaffected siblings	16	
Total	31	
Affected females	8	53
Affected males	7	47
Unaffected females	7	44
Unaffected males	9	56
Total	31	

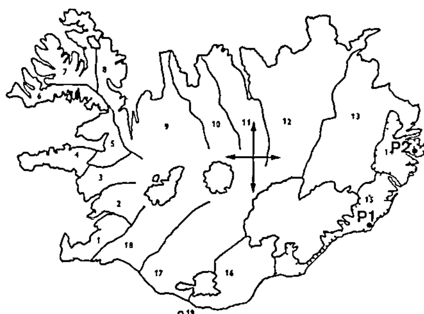


Fig 2 Map of Iceland Homesteads of the two branches of the Pelger family *P 1* detected in 1962 and *P 2* in 1975

particularly from the east of Iceland are among those approximately 40000 persons scanned for the anomaly (Table I)

The home counties of the Pelger families described have up to recent times been among the most isolated from the more densely populated part in the south west of Iceland due to distance and difficult communications The number of inhabitants in the two neighbouring counties was 4320 in Dec 1974 i e 2 83% of the total population of Iceland

Regional differences in frequencies of the Pelger anomaly in relatively isolated populations have been demonstrated by many authors and can in most cases be explained by genetic drift (5) or founder effect (12)

On the basis of results obtained it is considered probable that the Pelger anomaly gene has been present in Icelanders for over 200 years The scanning of blood films from approximately 20% of the population of Iceland makes it probable that the Pelger family described contains the only mutation of this kind in Icelanders

ACKNOWLEDGEMENT

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Studies on Hereditary Spherocytosis in Iceland

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ABSTRACT Thirty members with typical hereditary spherocytosis (HS) and over 90 apparently unaffected members belonging to 12 families have been studied Splenectomy has been performed on 22 HS patients Of nine HS individuals, who had not undergone surgical treatment in 1957, four suffered from temporary severe anaemia presumably due to aplastic crisis associated with influenza One of them died, a male 18 years of age Pedigree studies on one of the families indicate that the HS gene or genes have been transmitted through six generations over the past 200 years Marked deficiency in the number of affected compared with the apparently unaffected members in the HS families is present The most striking example of uneven genetic ratio is a sibship of 15 members investigated haematologically with one suffering from typical HS Much reduced penetration of the HS gene or the presence of the so-called "mild form" is upheld as the main explanation for the unevenness in the genetic ratio However, families are also present in which abortions and death at an early age indicated that selection against the affected could also disturb the genetic ratio in HS families

The population of Iceland which is Caucasian and mainly originates from Norway Ireland and Scotland (1) was nearly 219 000 in Dec 1975

Hereditary spherocytosis (HS) is the most common hereditary haemolytic disorder in Caucasian peoples with a roughly estimated prevalence of 1 5000 (13) Accordingly one would expect to find approximately 40 individuals with HS in the present population of Iceland In 1945–75 30 individuals with HS have been diagnosed three of whom have died in the 30 years covered by the present study

We have on record over 70 individuals with hereditary elliptocytosis which can be traced to a single mutation in the past (14) This haemolytic condition is thought at present to be the more common of the two

MATERIAL AND METHODS

Information on HS was collected by studying yearly reports and patient records of the three main hospitals in Reykjavik and through information from the main district hospitals outside the capital where surgery is undertaken Data were also obtained from the Department of Pathology University of Iceland Several of the HS cases were diagnosed in a private haematological laboratory for ambulatory patients (Domus Medica Reykjavik) Consultation and blood collections were also made by home visits in and outside Reykjavik Only HS cases considered to be reliably diagnosed in 1947–57 have been included in this study

Apart from information obtained from the sources mentioned above many Icelandic genealogical records have been indispensable for the pedigree studies

After 1958 standard haematological laboratory methods have been used (8) Older cases have been reinvestigated by these methods

Quantitative estimation of haptoglobin was performed by rocket immunoelectrophoresis (electroimmunoassay) as described by Laurell (15) Specific antiserum and control serum (normal range 57–356 mg/100 ml) were used purchased from Behringwerke Germany

Qualitative estimations were carried out by horizontal starch gel electrophoresis Buffer systems used were discontinuous Tris-citrate/borate (22) Staining with benzidine reagent according to Smithies (25) was applied These and other biochemical markers in the HS families will be published later

Pedigree studies

Seven HS families (nos 1–5 9 and 11) show the relationship between 23 affected family members (Figs 1–7) The families of seven HS cases have not been drawn up Available genealogical information on families 2 3 and 11 made it possible to draw up large pedigrees (Figs 5–7) which form a background for part of the material presented and a basis for continued research on HS in Iceland along with the smaller HS families found

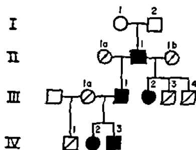


Fig 1 Family 1

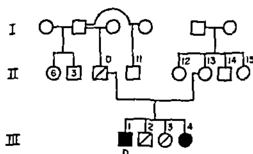


Fig 2 Family 4

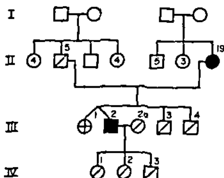


Fig 3 Family 5

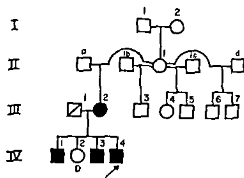


Fig 4 Family 9

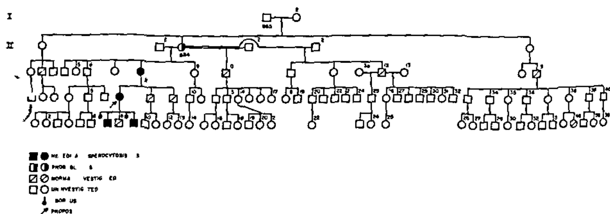


Fig 5 Family 2

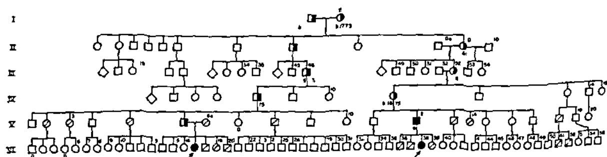


Fig 6 Family 3 Symbols as in Fig 5

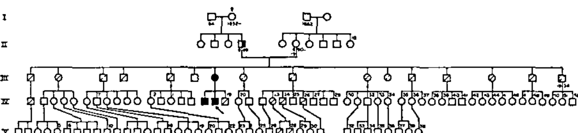


Fig 7 Family 11 Symbols as in Fig 5

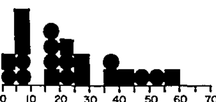


Fig 8 Age (y) of HS patients at the time of splenectomy Squares=males circles=females

CASE HISTORIES OF EIGHT SURGICALLY UNTREATED HS CASES

Case 13 (Fig 2 family 4 III 1)

Male born in 1938 admitted to a provincial hospital on Jan 4 1958 died two days later He had had an attack of abdominal pains and jaundice in 1956 considered to be due to gallstones

On Jan 3 1958 he had a high fever and looked pale Influenza was diagnosed and he was treated with aeromycin On Jan 5 his Hb was 10% Sahli he was breathless and had a temperature of 39°C He received a blood transfusion (500 ml) without improvement of the dyspnoea A further 250 ml of blood by slow drip made him worse and he developed respiratory distress Despite treatment with morphia and venesection he died

It is thought fairly certain that this young man had HS and his severe anaemia was caused most probably by an aplastic crisis induced by influenza (Tables I and II)

Case 15 (Fig 3 family 5 II 19)

Female born in 1905 with no history of jaundice or anaemia in either the patient or her five brothers three sisters or their parents Her husband has normal blood values and there was no history of anaemia or jaundice in his family In 1966 she had pains in the right upper abdomen (gallbladder?)

Haematological investigation in Feb 1966 12 5 g/100 ml haematocrit 36% MCHC 34 7% RBC 3 3 mill MCV 109 μ^3 WBC 3800 reticulocytes 2 3% bilirubin 2 2 mg/100 ml Spherocytes were seen in blood film She was considered to have mild HS

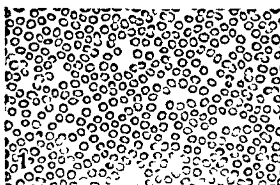
Case 20 (Fig 4 family 9 III 2)

Female born in 1945 HS diagnosed in Jan 1973 Haematological investigation Spherocytes present in blood film Hb 12 2 g/100 ml haematocrit 38% MCHC 32% WBC 4000 ESR 7 mm/h Normal differential count

She her husband and two of her sons were investigated because her youngest son IV 4 (propositus) had been found to have spherocytes and mild anaemia in Dec 1972 Her husband had normal blood film and normal haematological values She lost a daughter (Fig 4 family 9 IV 2) shortly after birth due to infection

Case 21 (Fig 4 family 9 IV 1)

Male born in 1962 HS was diagnosed in Jan 1973 Investigation Spherocytes present in blood film Hb 11 8 g/100 ml haematocrit 35% MCHC 34% WBC 6000 ESR 2 mm/h



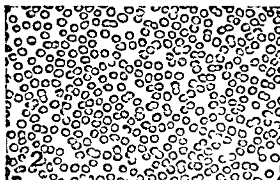


Fig 9 Blood films of members V 6 (*1*) and V 6a (*2*) of family 3

Case 22 (Fig 4 family 9 IV 3)

Male born in 1967 Diagnosis of HS made in Jan 1973 Investigation Spherocytosis present in blood film Hb 11 4 g/100 ml haematocrit 34% WBC 7000 ESR 3 mm/h Differential count showed eosinophilia (9%)

Case 23 (Fig 4 family 9 IV 4)

Male born in 1968 Mild anaemia and spherocytosis were found at routine blood investigation in Dec 1972 Observations Spherocytes and polychromasia in blood film Hb 106 g/100 ml haematocrit 30% MCHC 35% WBC 8900 ESR 3 mm/h These observations led to screening of the other family members for HS

Case 24 (family 10)

Male born in 1916 operated on for gallstones cholecystitis and jaundice in 1968 Haematological values in July 1975 Hb 14 4 g/100 ml haematocrit 41 0% MCHC 35 1% reticulocytes 2 0% total bilirubin 1 1 mg/100 ml ESR 5 mm/h WBC 4300 He had a moderate number of spherocytes in blood film and mild anisocytosis Diagnosis of mild HS was made He has not undergone splenectomy His gallbladder contained many small pigmented stones His son has HS (Table I 25/10)

Case 30 (family 12)

Female born in May 1974 When two weeks old she was admitted to hospital with anaemia Observation on admission Many spherocytes in blood film Hb 5 8 g/100 ml haematocrit 15% MCHC 38 1% WBC 12300 ESR 60/h bilirubin 1 0 mg/100 ml reticulocytes 25% thrombocytes 395000 haptoglobin 6 mg/100 ml Coombs test negative IgA 11 IgG 926 IgM 48 mg/100 ml Blood group O Rh D positive When three weeks old she had a blood transfusion packed red cells She had to be transfused again in July 1974 but during the following months and through 1975 she remained stable and kept her Hb at 12 g/100 ml Splenectomy was put off until she is 3 years of age

Her mother who has HS (Table I 29/12) was considered a sporadic HS case as her parents and three available siblings had no clinical or haematological signs of HS

RESULTS

The main clinical symptoms and signs and laboratory findings on 22 splenectomized HS patients are recorded in Table I Satisfactory results were obtained in 21 cases a girl of seven operated on in 1947 died two months after splenectomy The cause of death was uncertain The findings in 8 HS individuals who have not yet been splenectomized are summarized in the case histories above The approximate age of HS patients at the time of splenectomy is given in Fig 8

Four HS individuals whose spleen had not been removed suffered from aplastic crises and severe anaemia associated with the influenza epidemic in 1957 (Table II)

Over 90 apparently unaffected members in the 12 HS families studied have been investigated haematologically and clinically The results have been negative in most but equivocal in several cases Laboratory observations on 45 members of HS families 3 and 11 (Figs 6 and 7) are recorded in Table III Fig 9 shows blood films of two members of the former family

Results on the osmotic fragility tests of red cells in 3 members of family 11 (Fig 7) are shown in Table IV This family has also been selected to show the unevenness in the proportion of affected children in three generations (Table V)

Table I *Main symptoms signs and laboratory findings in 22 patients with HS before splenectomy*

	Case no /family no												
	1/1	2/1	3/1	4/1	5/1	6/2	7/2	8/2	9/2	10/3	11/3	12/3	14/4
Sex	♂	♂	♀	♀	♂	♀	♀	♂	♂	♂	♀	♀	♀
Age (y)	41	16	7	2	4	46	22	5	4	58	16	19	22
Anaemia	+		+	+	+	+	+	+	+	+	+		+
Jaundice	+		+	+	+	+	+	+	+	+	+		+
Gallstones										+			+
Splenomegaly	+		+			+			+	+	+		
Spherocytes	+	+	+	+	+	+	+	+	+	+	+	+	+
Reticulocytes	10 7			17 0	13 0	14 2		10 0		40 0	1 0	3 5	7 7
Hb (g/100 ml)	12 4		7 5	4 3	6 3	8 0		9 3	11 0	9 6	3 0	11 5	10 5
Bilirubin (mg/100 ml)				2 5		1 5		3 65	1 5	2 25		1 2	2 3
Coombs test				–	–	–							–
Osmotic fragility	+		+	+	+	+			+			+	+
Aplastic crisis						+					+		+
Weight of spleen (g)	925	*	255	80	170	580	300	300	160	775	1 700	250	180
Accessory spleen													

* Enlarged

Fig 10 Map of Iceland Geographic origin of 10 HS families indicated by asterisks and Arabic numerals

The geographical distribution of the families surveyed is indicated in Fig 10

DISCUSSION

Consequences of delayed splenectomy

As evident from Fig 8 which shows the approximate age at the time of splenectomy 15 of 22 HS patients underwent splenectomy after the age of 15 9 at 15–30 and 6 at 35–60 Six of the seven patients operated on before the age of 10 2 of whom were below 5 were all operated on during the last 15 years with satisfactory results (10)

5/5	17/6	18/7	19/8	25/10	26/11	27/11	28/11	29/12
	♀	♂	♀	♂	♀	♂	♂	♀
5	7	6	35	27	51	27	21	15
	+	+	+	+	+	+	+	+
	+	+		+	+	+	+	+
					+		+	
				+	+		+	
	+	+	+	+	+	+	+	+
5	10 3	11 0	7 0		12 4	13 3	22 0	
0	10 7	6 0	9 0	12 4	11 8	13 3	12 4	
7	4 8	2 1	1 2	3 4	1 9	3 8	6 4	
	–	–	–	–		–	–	–
		+	+					
150	340	200	290	1 285	600	470	1 400	375
	+		+			+	+	+

Table II *HS cases at risk during the influenza epidemic in 1957–58*

	Unoperated HS cases	Influenza aplastic crisis
Females	5	3
Males	4	1 †
Total	9	4

The delay in surgical treatment of HS cases has *admittedly led to serious consequences in four* Nine untreated HS cases were at risk when the so-called Asiatic influenza swept over Europe including Iceland in the autumn 1957 (24) The estimated morbidity was 40–50% but mortality was low As seen from Table II 4 of the 9 HS individuals who got the flu became seriously ill due to sudden severe anaemia presumably from aplastic crisis (21) This was confirmed by marrow biopsy in one case Three of these HS individuals were living in the same village on the north coast of Iceland A man of 18 died from a severe anaemic crisis (case 13)

In five of the 22 HS patients who underwent splenectomy two of them brothers accessory *spleen was found and removed There was no re*lapse due to any unremoved accessory spleen (6 16)

Table III *Laboratory findings in members of families 3 and 11*

Pedigree no	Age (y)	Sex	Condition[a]	Hb (g/100 ml)	PCV (%)	WBC	MCHC (%)	Reticulocytes (%)	Bilirubin (mg/100 ml)	Haptoglobin (mg/100 ml)	ESR
Family 3											
V 2	64	♀	N	15 7	46	4 800	34	1 0			47
V 3	62	♀	N	14 7	46	4 800	32	0.5			7
V, 5	59	♀	N	13 9	43	4 700	33	1 8			13
V 6	55	♂	N	15 3	44	9 550	35	2 42			14
V 6a	51	♀	N	14 1	39	3 750	36	3 6			6
V 8	51	♂	N	16 1	51	4 400	32	0 6			10
V 12	60	♂	HS	15 5	47						
V 13	71	♂	N	13 7	42 5	8 100	32 4	1 2	0.5	223	30
V 14	69	♀	N	14 7	43	6 000	34 1	2 8	0.5	260	33
V 19	60	♂	N	14 4	42 5		33 8	3 5	0 5	116	8
VI 16	28	♀	N	12 6	38	4 750	33	0 3			9
VI 17	29	♀	HS	11 5		10 250		4 9	1 2		7
VI 18	22	♂	N	14 0	43	5 700	33	0 9			3
VI 19	17	♀	N	12 3	38	5 800	32	1 0			12
VI 20	11	♂	N	13 8	41	5 700	34	1 3			6
VI 36	30	♂	N	14 9	47		31 7				
VI 37	38	♂	N	15 2	47 5		32 0				
VI 38	34	♀	HS	13 4	43		31 1				
VI 39	19	♀	N	13 1	40 5		32 3				
VI 51	40	♂	N	16 1	46	7 100	35 0	3 0	0 5	92	1
VI 52	34	♂	N	17 6	52	10 000	33 8	2 0	0 7	363	1
Family 11											
II 4	86	♂	N	14 7	43	5 300	34 1	1 8	0 5	55	8
III 1	61	♂	N	15 7	46 0	4 400	34 1	2 1	0 5	84	5
III 2	60	♀	N	13 2	39 0	3 400	33 8	1 5	0 5	130	5
III 3	58	♂	N	13 3	41 0	4 700	32 4	1 1	0 5	97	5
III 4	57	♂	N	14 4	41 5	4 300	34 6	1 3	0 5	101	4
III 5	56	♂	N	14.5	44 5	3 500	32.5	2.2	0 5	174	8
III 7	54	♀	HS	12 4	32 5	5 000	38 0	12 4	1 9	10	
III 8	53	♀	N	14 7	41 5	4 000	35 4	1 3	0 5	66	12
III 9	52	♂	N	15 3	49	5 000	31 2	2 2	0 5	67	4
III 10	51	♀	N	14 6	41	6 100	35 6	1 4	0 5	92	
III 11	49	♀	N	14 1	41	7 500	34 3	1 2	0 5	162	13
III 12	47	♂	N	14 5	43	7 100	33 7	1 6	0 5	122	17
III 13	46	♂	N	14 4	43 5	4 300	33 1	0 9	0 5	84	4
III 14	45	♀	N	14 5	42	5 800	34 5	1 1	0 5	74	5
III 15	44	♂	N	15 1	43	5 600	35 1	1 8	0 5	97	5
III 16	41	♂	N	15 2	43 5	6 700	39 9	1 4	0 5	92	10
IV 1	36	♂	N	14 9	42 5	7 800	35 0	1 2	0 5	111	3
IV 17	28	♂	HS	13 3	35 0	7 000	38 0	13 3	3 8	4	
IV 18	20	♂	HS	12 4	34 0	6 500	35 9	22 0	6 4		28
IV 23	29	♀	N	14 3	41	5 700	34 9	0 9	0 5	113	3
IV 24	26	♂	N	14 1	42 5	6 300	33 2	1 3	0 5	72	2
IV 26	24	♂	N	16 2	44 5	4 700	36 4	3 0	0 4	139	
V 27	10	♂	N	13 8	38 5	6 300	35 8	0 9	0 5	63	4
V 28	5	♂	N	13 1	36 5	6 800	35 9	1 1	0 5	69	2

[a] N=normal

Family studies of mild HS forms

It has for some years been thought improbable that the number of families with HS members indicates the same number of independent mutations of this kind in the small population of Iceland (12) During the 15 years of study an attempt has therefore been made to link up the families with HS members both genealogically and geographically The main results of these efforts are shown by pedigrees of families 2 3 and 11 In family 3 (Fig 6) common ancestors of overtly affected HS members have been found in two branches A firm relationship has not yet been established between families 2 and 11 (Figs 5 and 7) However some ancestors of

Table IV *Results of osmotic fragility tests (lysis %) in 3 members of family 11*

NaCl	Fresh blood (1/2 hour at 20°C) Father (II 4)	Son (III 15)	Daughter (III 8)	Control	Incubated blood (24 hours at 37°C) Father (II 4)	Son (III 15)	Daughter (III 8)	Control
0 30	100	100	100	100	100	100	100	100
0 35	80	100	100	90	100	100	100	100
0 40	35	50	25	6	100	100	100	92
0 45	0	10	0	0	93	100	92	45
0.50	0	0	0	0	30	40	29	10
0 55	0	0	0	0	10	12	7	0
0 60	0	0	0	0	8	3	3	0
0 65	–	–	–	–	–	3	3	0
0 75	–	–	–	–	0	0	0	0

these families have been traced to the same parish in a county in the south of Iceland where they lived in the first half of the last century Two independent HS mutations in a small parish is considered rather unlikely

Other smaller families have been traced to the district of origin according to information available on their parents and grandparents (Fig 10) As seen from the pedigrees and the clinical and haematological data presented the usual variants of HS families are represented families showing the dominant mode of inheritance and families with one affected member (sporadic) in which the presence of the so-called mild forms of HS is considered highly probable (4 5 7)

The latter type of HS is commented on by Dacie (7) as follows Subsequent writers have tended to overlook the possibility of the extremely mild forms described by Gänsslen For this form of variation within HS families family 11 (Fig 7) in the present survey appears to be of special interest Of 15 siblings investigated (III 1–5 and III 7–16) one has the typical form of HS and so have two of her three sons while all her brothers and sisters have haematological values within the normal range (Table V) Careful inspection of blood films revealed some spherocytes in several of the siblings and the father (II 4) The haptoglobin values (normal range 57–356 mg/100 ml) are within the normal range in 4 members and values within the lower normal range are found in the other 13 members investigated Red cell fragility on incubation for 24 hours showed moderately increased haemolysis compared with normal controls (Table IV) These findings are considered inadequate to discriminate affected from unaffected within sibship III 1–16 in family 11

The relative value of various tests for ascertaining genetics carriers of HS was assessed in a large scale study in 1962 (18) Discriminant ratios were calculated to be 88 1% when Hb reticulocytes bilirubin and spherocytes were used and rose to 89 6% when mechanical red cell fragility test was also performed The discriminatory power of these tests rose further to 91 4% when autohaemolysis with and without glucose and with adenosine was used Thus 4 tests accounted for 88% of the variability between normal subjects and classical HS cases whereas 13 tests accounted for 91% of the variability as stated by Mac Kinney et al (17 18)

Of 39 children in generation V family 11 two (V 29 30) are known to have had prolonged clinical neonatal jaundice One of them discharged on the 8th day mildly jaundiced The other had total bilirubin values of 13–14 mg/100 ml for 8 days after birth Rh and ABO incompatibility was not demonstrated as a cause Autohaemolysis of cord blood has been used to diagnose HS in the newborn and to discriminate between jaundice due to ABO incompatibility and HS in four patients with a family history of verified HS in one of the parents (9)

Another 2 members in generation V have been

Table V *Number of normal and overtly affected HS members of family 11*

Generation	Period	Normal	HS
I parents	1889–	1	1 (?)
II children	1912–	15	1
III grandchildren	1930–	52	2
IV great grandchildren	1950–74	39	
Total		107	4

investigated (Table IV) and show borderline changes pointing to a mild form of HS as was found in their mother IV 23 who was operated on be cause of gallbladder ailment at the age of 29 (Table III)

About 50% of newly born children with HS or elliptocytosis have prolonged jaundice in the neonatal period (20) Neonatal jaundice was present in 23 of 43 HS cases reviewed by Burman (3) in cluding identical female twins with HS studied by the author who found a negative family history for HS in about 40% of cases The dangers of kernicterus anaemia and postsplenectomy infection in infants are emphasized by Burman (3) and by Stamey and Diamond (26)

When the members of four generations belonging to family 11 (Fig 7 and Table III) are added up, half of the 107 individuals entered as normal or approximately 53 could theoretically be affected Only 3 of the four members listed as affected have been firmly diagnosed as suffering from HS and one (parent) is entered as probably affected

Admittedly only 24 of the 107 family members recorded in Table V have been studied haemato logically at this stage However the results ob tained so far (Table III) and discussed above illus trate the difficulties in ascertaining extremely mild cases of HS by laboratory methods available at resent

In family 3 (Fig 6) a common ancestry and estead have been established for the two ranches of the family which contain typically af fected HS members Pedigree studies indicate that the HS gene or genes could have been transmitted through six generations over the past 200 years In both branches some members (e g V 6 and V 19) have borderline findings (Table III) pointing to a mild form of HS The variations in haematological findings within families 11 and 3 are similar and suggest coexistence of the same HS gene in the strong and "mild form Although family 3 is from the north of Iceland and family 2 from the south (Fig 10) a distant relationship cannot be ex cluded

The present data suggest a much higher fre quency of HS gene or genes than is indicated by overtly affected HS cases admitted to hospitals The hospital frequency probably shows only the tip of the iceberg and not the true HS gene fre quency in the population This is thought to be mainly due to the presence of mild forms of HS which by a presently unexplained mechanism can occasionally cause severe typical forms of HS in one or more family units within the mild gene family A dominant mode of inheritance in HS has in general been agreed upon (2, 7, 23, 28 29) follow ing the report from Meulengracht (19)

Deviation from the expected genetic ratio often encountered in HS families was studied by Race (23) He showed that selection against the affected *in utero and early infancy could explain the un* evenness in the genetic ratio within HS families as well as the much reduced or absent penetration He also showed that the phenomenon of compensation i e parents making up for the loss of offspring by having unaffected children could further increase this unevenness These factors may still explain the preponderance of unaffected members in some HS families despite many significant changes in condi tions that have taken place since Race's material was compiled

Much needed tests for more direct ascertainment will hopefully evolve from the intensive studies in recent years on the red cell membrane in HS (11 12 13, 27)

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Leucocyte Migration Inhibitory Activity of Concanavalin-A-stimulated Lymphocytes

In vivo and in vitro Modifications with Dipyridamole and Acetylsalicylic Acid

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ABSTRACT Lymphocytes from 14 patients treated with a combination of acetylsalicylic acid (ASA) and dipyridamole (DIPY) showed a decreased ability to produce leucocyte migration inhibitory activity (LMIA) when stimulated with concanavalin A (Con A) The combined treatment also produced a decrease of leucocyte response to a standard LMIA containing culture supernatant Treatment with only one of the two drugs did not cause detectable alteration of the lymphocyte response to Con A or the leucocyte response to LMIA In vitro both DIPY and ASA were independently effective in decreasing the LMIA production of Con A stimulated lymphocytes and the leucocyte response to a standard LMIA containing culture supernatant

Lymphokines are released from sensitized lymphocytes stimulated with the specific antigen or from non sensitized lymphocytes stimulated with mitogens such as concanavalin A (Con A) or phytohemagglutinins (PHA) The lymphokines possess several biological activities such as leucocyte migration inhibitory activity (LMIA) chemotactic activity blastogenic activity cytotoxic activity capillary permeability increasing activity and others (16) Recently thrombocyte aggregating activity procoagulant activity and granulocyte fibrinolytic releasing activity have been described (4 5 6 14) The ability of lymphokines to produce vessel wall damage and their prothrombotic activity could partially explain the thrombotic process in the inflammatory damage of cell mediated immune reactions and the beneficial effect of thrombocyte antiaggregant and anticoagulant therapy (7 9 10 11 12 15)

In vitro drugs with thrombocyte anti aggregant activity such as dipyridamole (DIPY) acetylsalicylic acid (ASA) or its soluble derivative lysine acetylsalicylate (LASA) have a depressive effect on LMIA production by lymphocytes stimulated with Con A The same drugs in clinically relevant concentrations antagonize the LMIA effect (7 8) This could explain the activity of ASA and DIPY in clinical disorders with immunoinflammatory tissue damage since DIPY ASA and LASA could have a double role as modifiers of inflammation 1) the well known role as anti aggregants tending to limit thrombosis and 2) a role antagonistic to lymphocyte mediated tissue damage through a depressive action on lymphokines An important step to substantiate this theory would be the demonstration of a similar effect in vivo on lymphocytes and leucocytes during drug treatment of healthy subjects and patients with immunoinflammatory disorders

MATERIAL AND METHODS

Subjects and drug treatment

Venous blood lymphocytes were isolated from healthy male and female human adults and used for Con A induced production of LMIA rich and control supernatants as described elsewhere (3) These supernatants pooled and lyophilized were the standard LMIA preparations for all experiments Fourteen subjects male or female healthy or with immunoinflammatory diseases as presented in Table I were treated with one drug or drug combination

Table I *Data and examinations performed in 14 cases studied*

C=control ASA=acetylsalicylic acid DIPY=dipyridamole RA=rheumatoid arthritis H=healthy AA=allergic arthritis during Salmonella infection LED=disseminated lupus erythematosus

					Short term treatment				Long term treatment		
Case no	Sex	Age (y)	Diagnosis	In vitro	C	ASA	DIPY	ASA DIPY	C	ASA	ASA DIPY
1	♀	32	RA	+	+	+	+	+			
2	♂	33	H	+	+	+	+	+			
3	♂	33	H		+	+	+	+			
4	♀	68	H		+	+	+	+			
5	♂	32	H	+					+	+	+
6	♀	26	AA	+					+	+	+
7	♀	56	RA	+					+	+	+
8	♂	34	RA						+	+	+
9	♀	31	RA						+	+	
10	♀	22	RA						+	+	
11	♀	54	RA							+	+
12	♀	32	RA							+	+
13	♀	68	H							+	+
14	♀	28	LED	+							

Short term treatment The drugs were given in four daily doses at equal intervals Blood was drawn before treatment (control) and 1 2 4 12 24 and 48 hours after the beginning of treatment Sampling at 12 24 and 48 hours was performed 1 hour after drug intake The same subjects were tested both during ASA DIPY and combined ASA+DIPY administration The interval between each treatment period was 7 days

Long term treatment The drugs were given in four daily doses at equal intervals beginning with 72 hours of treatment and continuing for the following 72 hours i ASA+DIPY treatment Blood was tested before after ASA treatment and after ASA+DIPY In some subjects in vitro production and effect of LMIA and the in vitro modification of LMIA production and effect of ASA and LASA were examined The results were compared with the in vivo data Control subjects were tested in parallel with patients under study

Drugs For in vitro assays DIPY (Persantin® Boehringer Ingelheim Germany) and LASA (Aspegic® Egic Montargis France) were diluted/dissolved in tissue culture medium 199 (TC 199) with penicillin 67 IU/ml and streptomycin 67 μg/ml (Difco Laboratories Michigan USA) The concentrations employed were comparable with clinically relevant plasma concentrations as shown in Table II With respect to the salicylate levels obtained 0 9 g of LASA is equivalent to 0 5 g ASA and in the present report the concentration of LASA is calculated in ASA equivalents All drug preparations for in vitro experiments were stored at −20°C until use

For peroral administration the subjects were given coated Persantin tablets containing 25 mg DIPY each and tablets containing 500 mg ASA each (Magnyl Ph Nord) in daily doses of 8–10 and 50–60 mg/kg respectively All drugs were given at least 2 hours after the last meal and 1 hour before the next

Table II *Final concentrations (in vitro) of DIPY and LASA used in the experiments and clinically relevant plasma levels*

	Final concentration (μg/ml)	Relevant plasma concentrations during therapy (μg/ml)
DIPY	10	1–10
LASA	3	100–300

LMIA production

LMIA was obtained as previously described (1) Briefly peripheral blood mononuclear cells were incubated in the presence (active supernatants) or in the absence (control supernatants) of Con A 80 μg/ml (Pharmacia Uppsala Sweden) After 22 hours the supernatants were harvested and the control supernatants reconstituted with Con A Biologically active Con A as measured in the LMIA assay was then removed from the supernatant by passage through Sephadex G 100 columns (Pharmacia) The desalted eluates (three times the original sample volumes) were pooled and lyophilized Prior to assay they were reconstituted to the original volume in TC 199

LMIA assay

LMIA was measured with a modified indirect leucocyte migration agarose technique (ILMAT) as previously described (3) The LMIA was expressed as a migration index (MI)

$$MI = \frac{\text{mean migration area of cells in active supernatant}}{\text{mean migration area of cells in control supernatant}}$$

Assay for in vitro drug effect on leucocyte migration

Before migration 22×10^6 leucocytes were suspended in 90 μl TC 199 (control) and in 90 μl TC 199 containing drug After incubation at 37 C for 90 min the migration capacity of the leucocytes was measured by the ILMAT (22 h) (3) and the effect of the drug on the migration (MI_{drug}) was expressed as follows

$$MI_{drug} = \frac{\text{mean migration area of the drug treated leucocytes}}{\text{mean migration area of control leucocytes}}$$

Assay for in vivo drug effect on leucocyte migration

Venous blood was drawn before and after the drug treatment The migration areas of leucocytes from blood samples washed 3 times were measured and the effect of the drug on the leucocyte migration ($MI_{drug\ in\ vivo}$) was expressed as follows

$$MI_{drug\ in\ vivo} = \frac{\text{mean migration area of leucocytes after treatment}}{\text{mean migration area of leucocytes before treatment}}$$

Assay for in vitro drug effect on LMIA production

The ILMAT as described above was used and the drugs tested were included both in the Con A stimulated cell suspension and in the non stimulated control cell suspension during the entire incubation period The Con A preincubated and the Con A reconstituted supernatants were subsequently passed through Sephadex G 100 columns and examined for LMIA The calibration of the columns allowed only molecules larger than 10 000 daltons to be eluted thereby retaining DIPY and LASA The percentage influence of a drug on LMIA production was calculated on the basis of the MI obtained with culture supernatant from lymphocytes stimulated in the presence of drugs (MI_{drug}) and the MI in the parallel control without drug influence ($MI_{control}$) according to the formula

$$\%\ \text{inhibition}_{in\ vitro} = \frac{MI_{drug} - MI_{control}}{1 - MI_{control}} \times 100$$

Assay for in vivo drug effect on LMIA production

Similarly the percentage inhibition of drugs on LMIA production by lymphocytes during treatment was calculated on the basis of the $MI_{before\ treatment}$ and $MI_{after\ treatment}$ according to the formula

$$\%\ \text{inhibition}_{in\ vivo} = \frac{MI_{after\ treatment} - MI_{before\ treatment}}{1 - MI_{before\ treatment}} \times 100$$

Assay for in vitro drug modification of LMIA effect

After production of LMIA-containing culture supernatants and elution on Sephadex G 100 columns as described above the drugs were added to both the Con A-preincubated lymphocyte supernatants and the Con A reconstituted control supernatants so as to form the final concentrations indicated in Table II The LMIA was subsequently measured using the ILMAT (22 h) A drug s modification of the LMIA effect was calculated on the basis of the MI in the presence of the drug (MI_{drug}) and the MI in the absence of the drug ($MI_{control}$) according to the formula used above for calculating the in vitro drug influence on LMIA production

Assay for in vivo drug modification of leucocyte response to LMIA

The in vivo modification with drugs of the ability of leucocytes to respond to the standard" LMIA preparation (% inhibition of LMIA effect) was calculated on the basis of $MI_{before\ treatment}$ and $MI_{after\ treatment}$ using the formula indicated above for calculating the in vitro drug effect on LMIA production

Calculations

Each examination was made in quadruplicate and all MI values represent a mean of quadruplicates The general mean of the values observed in each group of subjects at the same stage of treatment was calculated The difference between the groups was evaluated by Wilcoxon s rank sum test

RESULTS

Drug effect on leucocyte migration

Treatment with DIPY and ASA did not induce any significant in vivo modification of leucocyte migration and comparable concentrations of DIPY and ASA did not induce any modification in vitro (Fig 1)

Effect of drugs on LMIA production

In vitro both DIPY and LASA in concentrations comparable with those obtained in the blood during treatment caused significant inhibition of LMIA production In vivo the LMIA production was not modified during treatment with one drug Combined therapy for 24 hours however significantly decreased the ability of lymphocytes to produce LMIA under Con A stimulation (Fig 2) In one case with an initial pretreatment MI of 0 59 DIPY was continued after 3 days of ASA+DIPY treatment (MI 0 96) and the treatment was then con

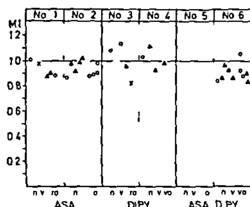


Fig 1 In vitro and in vivo influence of drugs on leucocyte migration *ASA*=acetylsalicyl c acid *DIPY*=d pyrida mole ▲ healthy subjects O patients with rheumato d arthritis ● patient with allerg c arthrit s during Salmo nella infection ×=patient with d sseminated lupus erythematosus *MI* m gratio~ dex The differences between groups are not s gnificant

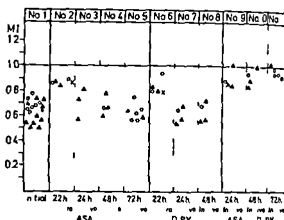


Fig 3 In vitro and in v vo influence of drugs on leucocyte abil ty to respond to a standard LMIA Symbols and abbreviations as in Fig 1 S gnif cance of differences be tween groups as in F g 2

tinued for two weeks with only ASA A new assay of LMIA production showed a recovered ability of lymphocytes to produce LMIA (MI 0 68)

Drug modification of the ability of leucocytes to respond to LMIA

When leucocytes were incubated in vitro with either DIPY or LASA their ability to respond to a stan dard LMIA preparation was depressed in both ases (Fig 3) However treatment with one of the did not decrease leucocyte reactivity to

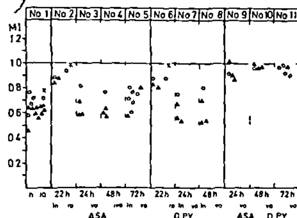


Fig 2 In vitro and in vivo influence of drugs on lymphocyte ab l ty to produce LMIA after Con A stimulation Symbols and abbreviations as n Fig 1 The following differences between groups are s gn f cant No 1 No 2 No 1 No 6 No 1 No 9 No 1 No 10 and No 1 No 11 ($p<0\ 01$) No 3–No 9 No 4–No 10 No 5–No 11 No 7 No 9 and No 8–No 10 ($p<0\ 05$)

LMIA Only leucocytes from patients treated simultaneously with both DIPY and ASA were in hibited in their response to LMIA The removal of DIPY after three days of combined ASA+DIPY treatment again permitted the leucocytes to respond to LMIA despite continuation of the ASA treat ment (MI before treatment 0 70 after ASA+DIPY treatment 0 90 after 2 weeks of treatment w th only ASA 0 80)

DISCUSSION

In vitro DIPY as well as LASA in therapeutically relevant concentrations decrease both the Con A induced LMIA production and the LMIA effect on migrating cells (7) In the present study the plasma level of DIPY during treatment was 2–6 μg/ml and that of ASA 100–180 μg/ml In vitro these concen trations were sufficient for a depress ve effect As to LASA even concentrations 100 t mes lower than the cl nically relevant were active However con centrations usually obtained during ASA therapy significantly decreased the migration areas of ind cator cells The concentrations d d not affect the cell viability when tested in the trypan blue exclu sion test Furthermore pulse treatment of leucocytes with 100 μg/ml of LASA for 1 hour did not cause inhibition of leucocyte m gration (Unpub lished data)

The in vitro effects on leucocytes and lymphocytes could not be demonstrated during treatment

with either DIPY or ASA alone only during simultaneous treatment with both drugs This difference between in vitro and in vivo effects may have several explanations It must be emphasized that the in vitro experiments were carried out in a serum free system and with equilibrated and controllable drug levels without metabolic modification as in vivo Since both ASA and DIPY (13) have a high affinity for plasma proteins the in vivo protein binding may decrease their effect by lowering the free active fraction of the drugs

DIPY and LASA have a transitory effect on lymphocytes and seem to act directly on cells since they do not inactivate the mitogens which after incubation with the drugs keep their ability to stimulate lymphocytes (8)

An other indication of a cell-directed action of the drugs is the fact that ASA and DIPY in vivo produce cell membrane modifications by a decrease of B lymphocytes as detected by immunospecific staining of surface immunoglobulin with fluorescein-conjugated rabbit antihuman immunoglobulins (8) The B lymphocyte population is partially restored after 24 hours of cell culture in drug free condition

These in vivo experiments associated with the in vitro assays (7) show that DIPY and ASA can modify lymphocyte functions probably by inhibiting lymphokine production and by modifying lymphokine effects The combined use of the two drugs detectably decreases the capacity of lymphocytes in vivo to exert biological activity of presumed importance in immunological inflammation associated with type IV reactions A similar in vivo effect during treatment with one drug only cannot be excluded but is not detectable with the present system The data thus give experimental support for the reported effect of anticoagulant antiaggregant therapy in cell mediated immune reactions (10 11 12 15)

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Treatment of Progressive Systemic Sclerosis (Scleroderma, PSS) with a New Drug Influencing Connective Tissue

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ABSTRACT Cyclofenil is a new diphenyl ethylene derivative related to stilboestrol without oestrogenicity but with marked effects on connective tissue metabolism The drug has been tested, in a daily dose of 200 mg×3, in six patients with progressive systemic sclerosis (PSS) to analyze the expected beneficial effects on the PSS symptoms The typical skin hardness joint and muscle rigidity, and reduced breathing capacity were improved to varying degrees The only side-effect was a slight transient liver enzyme elevation in 1 out of 6 patients A slight increase was found in urinary calcium and hydroxyproline excretion In several cases serum calcium cholesterol, triglyceride and in some cases the serum uric acid levels were decreased The ANF titres diminished to varying degrees in 4 out of 6 patients These results indicate that further detailed clinical and laboratory studies on the therapeutic potential of cyclofenil in PSS and other diseases affecting connective tissue seem to be justified

Progressive systemic sclerosis (PSS) is a severe chronic disease involving the connective tissues of the body The etiology is essentially unknown Defects in the collagen metabolism and autoimmune changes in various organs have however been studied and demonstrated by several authors (3 11 14 21 22)

In the recent 15 years several types of pharmacological agents have been tried as therapeutic drugs involving several mechanisms of action Among these prednisolone selected gestagens and D-penicillamine induced amelioration of the disease and some of them are employed at present although side effects may complicate their use

Animal experiments including biochemical (7 9) and pharmacological (6 8) studies testing several hormones have been reported For clinical use potent oestrogens weak oestrogens and antioestrogens are of particular interest The compounds elicit a marked decrease in the rate of sulphate incorporation into different connective tissues Furthermore a recently developed in vitro method demonstrated definite effects of antioestrogens and some of their derivatives on mucopolysaccharide metabolism in several types of target cells (Herbai in preparation for publication) Having compared the relation between weak oestrogenicity and the effects on connective tissue it was found that cyclofenil (Sexovid® Ferrosan (NFN)) exhibited a marked influence on connective tissue without toxic effects (7 9)

Cyclofenil was first introduced in an attempt to treat a very severe PSS case (10) The result was a marked improvement which has persisted during 3 years continuous administration of cyclofenil After these promising results we extended our studies to other PSS patients This paper is a preliminary report of the most relevant biochemical and clinical results to date

MATERIAL AND METHODS

Six PSS patients were thoroughly investigated by means of endocrinological chemical and some immunological laboratory tests Cyclofenil was given orally to all in a dose of 200 mg 3 times a day The personal data duration of PSS signs before the start of the treatment and length of the cyclofenil administration are shown in Table I Each patient was hospitalized 4 times a year for laboratory and clinical controls

The chemical structures of cyclofenil (developed and marketed by AB Ferrosan Malmo Sweden) and its primary source stilboestrol are shown in Fig 1

Table I *Review of patient data duration of PSS before treatment and duration of treatment*

Pat no	Sex	Age (y)	Occupation	Duration of disease at start of treatment (y)	Length of cyclofenil administration (mo)
1	♀	67	Laundry assistant	13	5 5
2	♂	68	Bricklayer	1 5	3
3	♂	48	Glazier	10	7
4	♂	57	Foundry worker	5	36
5	♀	29	Factory worker	1	8
6[a]	♂	41	Miner	10	12

[a] Mother died of very severe PSS

RESULTS

Serum calcium and cholesterol values showed a slight decrease and serum triglycerides became markedly lower during the cyclofenil treatment (Table II) The serum cholesterol lowering effect of cyclofenil had been observed previously in rats and mice fed cholesterol rich diets (Herbai unpublished observations)

The urinary calcium and hydroxyproline excretions were considerably increased in some of our treated patients In 4 of the 6 cases serum uric acid values decreased during the study A similar tendency after high doses of powerful oestrogens has been reported in a study on 22 male prisoners convicted of sexual offences (17) The antinuclear factor (ANF) titre decreased in 4 out of 6 patients and was unchanged in 2

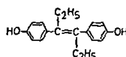
The clinical effects of the treatment are given in Table III in terms of a simple arbitrary 5 point scale Some of the patients particularly the younger were greatly improved and most of them obtained some benefit from the treatment A more detailed presentation of the results with regard to joint motility and pulmonary function will be published shortly

The side-effects observed included a bleeding resembling menstruation in a 67-year old woman which was treated by curettage and a slight transient elevation of SGOT (ASAT) and SGPT (ALAT) in 1 patient

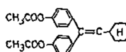
DISCUSSION

Many types of pharmacologic agents have been tested in the treatment of scleroderma (Table IV) Vasoactive drugs only produced a locally increased skin circulation in finger tips and toes Corticosteroid therapy is of little value because only the early oedematous phase of the disease can be temporarily alleviated Azathioprine and chlorambucil frequently cause blood cell damage when used as a long term therapeutic agent Among the clinically tested drugs D penicillamine seems to ameliorate several organ changes by diminishing the amount of collagen and reducing the biosynthesis of hydroxyproline (5, 11, 22, 23) Severe side-effects such as fever allergic skin rashes kidney and liver damage and rarely optic neuritis compel termination of the treatment An attempt with gestagen treatment with relatively good effects was reported recently (19) Side effects such as weight gain water retention vomiting and uterine bleeding occurred in some patients

The very promising results in one of our patients treated continuously with cyclofenil for three years (10) (no 4 in the present study) motivated us to extend the use of this drug to several other patients



diethylstilboestrol NFN (Stilbol®)



cyclofenil NFN (Sexovid®)

Fig 1 Chemical structure of stilboestrol (trans 3 4-bis(4 hydroxyphenyl) 3 hexen) and its antioestrogenic (15) derivative cyclofenil (bis (p acetoxyphenyl)cyclohexylidene methane)

Table II *Comparison of some laboratory data recorded before and during cyclofenil treatment*

The first figure is the pretreatment value and the second the value recorded after 3 months of treatment or at the last control

Pat no	Serum calcium (mmol/l)	Urinary calcium/24 h (mmol/l)	Serum cholesterol (mmol/l)	Serum triglyceride (mmol/l)	Serum uric acid (μmol/l)	ANF	Urinary hydroxy proline (mg/24 h/m²)
1	2 32 2 18	1 20 1 75	3 40 3 13	1 13 0 96	380 220	1/800 1/400	6 10 12.50
2	2 35 2 35	1 90 1 40	5 30 3 80	0 99 1.34	320 396	1/10 Neg	26 90 24 0
3	2 48 2 32	3 50 5 50	5 51 4 40	1 48 0 85	417 365	1/100 1/25	13 80 19 40
4	2 45 2 30	4 85 10.50	6 20 4.30	2 20 1 06	420 315	1/400 Neg	9 30 25 20
5	2 40 2 26	4 75 5 75	5 05 3.34	1 95 0 88	535 196	Neg Neg	1 22 50 20
6	2 45 2 41	3 00 4 20	6 00 6 80	2 03 3 40	265 329	1/25 1/25	4.50 7 0

As shown in Table I the duration of cyclofenil treatment to date ranges from 3 months to 3 years In all our cases the signs of remission appeared after 1–2 months

Previous experimental studies have demonstrated that the connective tissue activity of cyclofenil is much stronger than its oestrogenicity when compared with other oestrogens (7) In a recently published very detailed review on oestrogens and antioestrogens (15) cyclofenil was classified as an antioestrogenic compound We have treated 4 men with cyclofenil and no feminizing effects were observed However after improvement of scleroderma an increased sexual potency was noticed in two males (Table III) The slight elevation of SGOT (ASAT) and SGPT (ALAT) in 1 patient normalized rapidly after withdrawal of the drug for two months and remained normal when the treatment was recommenced with a lower dosage (100 mg× 3 daily)

It has been suggested (20) that the development of scleroderma might be related to exposure to silica dust in mines foundries cement factories potteries brick yards and glass factories From this point of view it is of interest that 5 of our 6 patients had one of these occupations (Table I) Some chemical agents in silica dust may thus initiate immunological reactions causing the severe connective tissue and collagen damage

The changes elicited in calcium and lipid metabolism by potent oestrogens have been reported previously The present results suggest that the antioestrogenic cyclofenil may probably be of some benefit in other types of connective tissue and metabolic diseases as well

ACKNOWLEDGEMENT

The experimental studies and clinical investigations were supported financially by Ferrosan Malmö Sweden

Table III *Clinical effect of cyclofenil treatment and side effects observed*

The degree of improvement is given according to an arbitrary 5 point scale graded from + (slight relief) to +++++ (very good improvement)

Pat. no	Skin hardening and circulatory function	Joint rigidity and flexibility	Lung function and breathing capacity	Side-effects
1	+	+	+	Uterine bleeding for 1 week curettage with good effect
2	+++	++	+	
3	+++++*	++++	+++++	
4	++++	+++	++	
5	+++++	++++	+++	Slight elevation of SGOT and SGPT (2 mo)
6	+++	++	++	

Increased sexual potency after the treatment
* Gangrenous ulceration of the finger tips disappeared

Table IV *Review of drugs either used or tested in the treatment of PSS during the recent 15 years*

Pharmacological agent	Reference no
Adrenal corticosteroids (prednisolone)	4
Vasoactive drugs phenoxybenzamine (Dibenzyline) dipyridamole (Persantin) nicotinic acid procaine	24
Anti inflammatory and experimental drugs potassium p aminobenzoate salicylate dimethyl sulfoxide disodium edetate (EDTA)	24
D-thyroxine	1
Benzyl penicillin-diethylamino ethyl ester	18
Low molecular weight dextran (Rheomakrodex®)	12
Immunosuppressive drug (azathioprine Imurel®)	13
Chlorambucil (Leukeran®)	16
Gestagen hormones norethisterone (Primolut Nor®) hydroxyprogesteron capronate (Proluton-depo®)	19
Hydralazine	2
D-penicillamine (Cuprimine®)	5
	23
	11
Stilboestrol derivative (anti oestrogen) cyclofenil	10

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Giant-cell Arteritis, Temporal Arteritis and Polymyalgia Rheumatica

A Retrospective Study of 63 Patients

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ABSTRACT The initial clinical symptoms, the course of the disease and the effect of corticosteroid treatment have been analyzed in a retrospective study of 63 patients with temporal arteritis or polymyalgia rheumatica The relationship between the physical examination of the temporal regions, the ophthalmological examination, and biopsy from the temporal artery with respect to the diagnostic value were examined Histological examination of biopsy specimens from the temporal artery in 58 patients revealed arteritis in 46 Half of the patients had only local symptoms from the temporal regions, one fourth presented such symptoms as well as myalgias, and one fourth had myalgias only Patients presenting local symptoms of temporal arteritis as well as of myalgias had always had myalgias as the initial symptom and developed local symptoms of temporal arteritis 1–24 months later Permanent reduction of vision occurred in 20% of the patients Symptoms of generalized arteritis were observed in several patients The overlapping of the clinical symptoms, the positive biopsy findings in patients with polymyalgia rheumatica as the only local symptom and the identical reaction to corticosteroid treatment support the conception of temporal arteritis and polymyalgia rheumatica as two manifestations of the same disease The physical and the ophthalmological examinations were of limited diagnostic value Positive biopsy findings were seen in 25 patients with normal palpatory findings, and in 46 patients without eye symptoms the ophthalmoscopic examination revealed no signs of arteritis If the first biopsy from the temporal artery is negative, biopsy from the contralateral temporal artery should be performed Correctly timed corticosteroid treatment in adequate doses can prevent reduction of vision in giant-cell arteritis The treatment is a long term therapy, its average duration in the present study being more than two years

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Temporal arteritis has been recognized as a clinical syndrome since Horton et al in 1932 reported two cases (18) The classical symptoms are systemic manifestations such as fever malaise and weight loss The local symptoms are confined to the temporal regions and include pain and scalp tenderness The temporal arteries may be thickened and tender with diminished pulsation on palpation (2 18)

Polymyalgia rheumatica (4) has been known under various names as a condition which mainly occurs in elderly persons and is clinically characterized by pain and stiffness in the region of the neck and shoulders the lumbar region the buttocks and the thighs accompanied by constitutional symptoms (4 6 10 11 17 22 23 24 26 32)

In recent years the relationship between the two syndromes has been frequently discussed In all probability temporal arteritis and polymyalgia rheumatica are two cardinal manifestations of a generalized arteritis which may involve the aorta and the large and middle sized arteries (8 11 12 13 14 28 30 32) The arteritis has been classified as a variant of polyarteritis (33) and the name giant-cell arteritis has been suggested (9)

The present report comprises a retrospective investigation of 63 patients with clinical symptoms of temporal arteritis or polymyalgia rheumatica It analyzes the variations in the initial symptoms the course of the disease and the effect of corticosteroid treatment At the same time we evaluated the relationships between the physical examination of the

Table I *Relations between local symptoms of temporal arteritis and polymyalgia rheumatica and biopsy findings in 63 patients*

Local symptoms of temporal arteritis (TA) comprising local palpatory findings and/or temporal headache

	Biopsy findings			
	Positive	Negative	No biopsy	Total
Local symptoms of TA without myalgia	21	1	0	22
Local symptoms of TA and myalgia	12	0	1	13
Myalgia without local symptoms of TA	9	11	4	24
Neither local symptoms of TA nor myalgia	4	0	0	4
Total	46	12	5	63

temporal regions the ophthalmological examination and biopsy from the temporal artery with respect to diagnostic value

PATIENTS AND METHODS

The criteria for including the patients in the retrospective study were (*a*) histological evidence of giant-cell arteritis and/or (*b*) local symptoms of temporal arteritis and/or polymyalgia rheumatica in patients more than 50 years of age with an ESR above 50

In the 10-year period 1964–73 63 patients fulfilling these criteria were seen in Medical Department C Gentofte Hospital Copenhagen The patients were 55 women and 8 men with an average age at the time of diagnosis of 71 years (range 49–80) Physical examination with special attention to the temporal regions was carried out in all patients Ophthalmological examination was performed in 59 patients in the Department of Ophthalmology Gentofte Hospital

Laboratory examinations included ESR plasma proteins (haptoglobin albumin α β and γ globulin) AST RAT Rose Waaler ANA and Hb In all patients an attempt was made to obtain biopsy specimens from the temporal artery In the presence of local symptoms of temporal arteritis in one of the temporal regions the biopsy specimen was taken from that side Otherwise the site of the biopsy was chosen at random The histological studies were carried out in the Laboratory of Rheumatology or in the Institute of Pathology Gentofte Hospital The specimens were examined by routine methods and no serial sections were made Muscle biopsy from the temporal deltoid or vastus lateralis muscle was performed in the majority of the patients

RESULTS

Cardinal symptoms

All patients complained of systemic symptoms su as fatigue malaise or anorexia 46 had experien prolonged fever with a duration of 2 weeks- months 17 reported a weight loss of more than 3 and 16 had transient arthralgias The relations tween the local cardinal symptoms of temporal teritis and polymyalgia rheumatica and the bio findings are shown in Table I Either one or b local cardinal symptoms had frequently been pr ent for a long period before the diagnosis was est lished Thirty five patients had had local sympto of temporal arteritis for an average period o months (range 1–24) and 37 had suffered from my gias with an average duration of 7 months (ra 1–18) Patients presenting with local symptoms temporal arteris as well as myalgias invariably h myalgias as the initial symptom and developed lo symptoms of temporal arteritis 1–24 months lat Four patients had neither local symptoms of tem ral arteritis nor myalgia Their symptoms includ fever, anaemia elevated ESR and weight loss

The results of the physical examination of t temporal regions are given in Table II It appe that more than half of the patients with histologi evidence of temporal arteritis had normal palpato findings Of 12 patients with negative biopsy 11 h normal palpatory findings whereas in 1 patient pulsation could be recognized in one of the temp ral arteries

Ocular symptoms

Permanent reduction of sight occurred in 13 patien (20%) visual impairment being the initial sympto in 3, in 10 patients the sudden onset of blindne was preceded by local symptoms of temporal art ritis for a period of 1 week–3 months Several p tients had noted episodes of transient blurring vision or flittering scotomata up to 3 weeks befo

Table II *Local palpatory findings in 46 patien with histological evidence of temporal arteritis*

Palpatory findings		No of pats
Normal		25
Pathological		21
No pulsation of artery	9	
Firm nodular artery	10	
Tenderness on palpation	15	

Table III *Visual impairment in relation to local symptoms of temporal arteritis and polymyalgia rheumatica in 63 patients with giant cell arteritis*

Local symptoms of temporal arteritis (TA) comprising local palpatory findings and/or temporal headache

	Unilateral	Bilateral	Total
Local symptoms of TA without myalgia	3	4	7
Local symptoms of TA and myalgia	5	1	6
Myalgia without local symptoms of TA	0	0	0
Neither local symptoms of TA nor myalgia	0	0	0
Total	8	5	13

Table IV *Clinical symptoms of giant cell arteritis apart from temporal arteritis and polymyalgia rheumatica in 63 patients*

	No of pats
Depression	2
Dementia and mental confusion	3
Impairment of consciousness	2
Vertigo	3
Blindness	13
Ophthalmoplegia	1
Acute loss of hearing	1
Aortic arch syndrome	1
Hepatic artery involvement	1
Mesenteric giant-cell arteritis	1
Intermittent claudication	1
Arthralgia	16

permanent loss of vision Five patients developed bilateral blindness In 4 of them the bilateral involvement appeared simultaneously in the fifth patient with a time-difference of 4 days between the unilateral symptoms All patients who developed visual impairment had positive biopsy findings and no patient with negative biopsy findings had ocular changes

Two patients became blind during treatment with corticosteroids In both patients however the glucocorticoid doses were small (equal to 2 5 mg prednisone per day) and insufficient to suppress the disease activity reflected in a marked elevation of ESR No patients developed loss of vision during treatment with corticosteroids in adequate doses Corticosteroids had no effect on established blindness In Table III patients with visual impairment are grouped according to the presence of local symptoms of temporal arteritis and myalgias All 13 patients who developed visual impairment had local symptoms of temporal arteritis and 6 of them also had myalgias In these patients myalgia was the initial symptom and the only manifestation of the disease during an average period of 6 months After the onset of local symptoms of temporal arteritis blindness occurred within a few weeks Blindness did not occur in any patient with myalgia as the only cardinal symptom

Ophthalmoscopic examination in 13 patients with visual impairment showed ischaemic papilloedema (ischaemic optic neuritis) in 7 occlusion of the central artery in 4 and no changes in 2 patients In 46 patients without eye symptoms the ophthalmoscopic examination revealed no signs of arteritis

Other clinical symptoms of giant cell arteritis

Apart from the local symptoms of temporal arteritis and polymyalgia rheumatica a number of other symptoms reflecting the generalized nature of the arteritis were registered (Table IV) Neurologic disturbances were frequent during the course of the disease The symptoms improved or disappeared after the start of corticosteroid therapy One patient had an aortic arch syndrome Arteriography revealed stenosis of the right subclavian artery and complete occlusion of the left subclavian artery The left vertebral artery showed segmental narrowing and there was no filling of the right vertebral artery The aortic arch and the carotid arteries appeared normal Mesenteric giant cell arteritis was observed in one patient who developed intestinal obstruction on the day on which the diagnosis of giant cell arteritis was established by biopsy from the temporal artery At laparotomy the small intestine showed a gangrenous segment and histological examination of the resected intestine revealed mesenteric giant-cell arteritis After the operation the patient developed a diffuse peritonitis and anuria and died

Laboratory findings

All patients had a considerably elevated ESR (Table V) Similarly the other two phase reactants plasma fibrinogen and α_2 globulins were generally elevated A comparison between the median values of the ESR plasma fibrinogen and α_2-globulin in patients with histological evidence of giant-cell arteritis and in those with negative or no biopsy showed

Table V *Laboratory findings in patients with giant cell arteritis*

	Median	Range	Normal range
ESR (mm/h)	93	45–147	2–10
Plasma fibrinogen (g/100 ml)	0 66	0 38–1 27	0 24–0 40
α_2 haptoglobin (g/100 ml)	3 93	2 48–5 05	0 4–1 4
C reactive protein	31	20–50	0–5
Plasma electrophoresis *(g/100 ml)*			
Albumin	3 3	2 0–4 3	3 9–4 7
α_1 globulins	0 5	0 2–0 8	0 3–0 5
α_2 globulins	0 9	0 6–1 4	0 3–0 6
β globulins	0 9	0 5–1 4	0 4–0 7
γ globulins	1 3	0 6–2 1	0 7–1 3
Hb (g/100 ml)	11 2	7 2–14 4	11 7–15 7

no differences In general AST, RAT Rose Waaler and ANA as well as the LE cell test were negative

Biopsy findings

Representative biopsy specimens from the temporal artery were available in 58 of the 63 patients not available in 5 either because the specimen did not contain any artery or because the patients refused diagnostic biopsy The results of the histological examination of the specimens are listed in Table VI Biopsy from the contralateral artery was performed in 13 patients in whom the first specimen was normal In 7 of these patients the second biopsy showed arteritis Muscle biopsy was performed in 35 patients, 29 of whom had myalgias Microscopic examination of the specimens was normal (29 patients) or revealed only minor non specific changes (6 patients)

Table VI *Result of examination of biopsies from temporal arteries in 58 patients with clinical symptoms of giant cell arteritis*

	No of pats
Positive biopsy	
First	39
Second contralateral (first biopsy negative)	7
Total	46
Negative biopsy	
First	6
From both sides	6
Total	12

Table VII *Effect of corticosteroid therapy on ESR in 26 patients with giant cell arteritis*

	After 1 week	After 2 weeks	After 3 weeks	Total within 3 weeks
Decrease to values below 50% of the initial	10	13	3	26
Decrease to normal values (<15 mm/h)	1	12	5	18

Treatment course and prognosis

Glucocorticoid treatment was started as soon as the diagnosis of giant-cell arteritis had been established The initial dose of prednisone was 30–60 mg/day Dramatic improvement in the clinical symptoms was evident within a few days after the start of treatment In 26 patients ESR was checked at least once a week during the first weeks of corticosteroid treatment (Table VII) In all of them the ESR fell to values below 50% of the initial within 3 weeks The patients were controlled at regular intervals in the Out patient Department The glucocorticoid doses were gradually reduced the level being determined by the lowest dose which kept the patients free from clinical symptoms and the ESR normal At the time of evaluation it had been possible to withdraw the corticosteroid treatment in 26 patients without a flair up in disease activity The average duration of treatment in these patients was 2 years (Table VIII) similar in patients with temporal arteritis and polymyalgia rheumatica

At the time of evaluation 26 patients were still on medication The observation time ranged from 4 to 84 months (mean 24) In 10 patients the treatment had been discontinued (4 died 3 had been transferred to other hospitals 3 discontinued on their own request)

Out of the 4 deaths during the observation period only one was due to arteritis as mentioned above

In a few patients we observed rather severe complications to corticosteroid therapy Two patients were admitted to hospital with a perforated peptic ulcer and one patient developed necrosis of the femoral head One patient had meningitis caused by Listeria monocytogenes

Table VIII *Duration of corticosteroid therapy in relation to local symptoms of temporal arteritis and polymyalgia rheumatica in 26 patients*

Local symptoms of temporal arteritis (TA) comprising local palpatory findings and/or temporal headache

	No of pats	Duration of treatment (mo)	
		Mean	Range
Local symptoms of TA without myalgia	8	24	4–56
Local symptoms of TA and myalgia	6	28	16–48
Myalgia without local symptoms of TA	11	21	6–46
Neither local symptoms of TA nor myalgia	1	56	56
Total	26	26	4–56

DISCUSSION

When evaluating the results of the present analysis allowance must be made for the retrospective character of the study Giant-cell arteritis is seen almost exclusively in patients more than 50 years old (1 11 12) We observed a predominance of women a phenomenon also noticed by others especially in patients with polymyalgia rheumatica (5 6 12 24 28) The simultaneous occurrence of the local cardinal symptoms of polymyalgia rheumatica and temporal arteritis the positive biopsy findings in patients with polymyalgia as the only local symptom and the identical reaction to treatment with corticosteroids are consistent with the conception of temporal arteritis and polymyalgia rheumatica as two manifestations of the same disease a generalized arteritis (1 8 11 12 28 32) In the present investigation all the patients who presented local symptoms of polymyalgia rheumatica as well as of temporal arteritis had myalgia as the initial symptom Paulley and Hughes (28) suggested that polymyalgia rheumatica is a prodromic manifestation of temporal arteritis Since then other investigators have demonstrated the high incidence of giant cell arteritis in arterial biopsies from patients with symptoms of polymyalgia rheumatica but without local symptoms of temporal arteritis (1 8 12 13 32)

Blindness occurs in 30–50% of patients with untreated giant-cell arteritis (2 11 15 16 23 31) In our series 13 patients (20%) developed permanent reduction of sight Blindness did not occur in any patient with myalgias as the only cardinal manifestation It must be born in mind however that these patients were treated with corticosteroids on the basis of the diagnosis of polymyalgia rheumatica The spontaneous course of the disease in these patients would probably have been similar to that in those with both myalgias and local symptoms of temporal arteritis The 13 patients with local symptoms of polymyalgia rheumatica as well as of temporal arteritis all had myalgias as the initial symptom and 6 of them developed visual impairment only a few weeks after the onset of the local temporal symptoms

Arteritis localized to the carotid arteries the vertebral arteries and the intracerebral arteries has been demonstrated by angiography (7) and at autopsy (5 9 21) This involvement may cause symptoms of diffuse or focal cerebral ischaemia (15 16 21) It is important to recognize neurological and psychiatric symptoms as a possible manifestation of giant-cell arteritis as the symptoms may be reversible on corticosteroid treatment (16 21 28) As another sign of the generalized nature of the arteritis we observed involvement of the major branches of the aorta the intestinal blood vessels and the arteries of the lower limbs similar to the findings reported by others (1 8 11 12 14 20 21 28)

Marked alterations in acute phase reactants especially a pronounced elevation of ESR are characteristic for giant cell arteritis (1 3 6 8 10 11 25 31 32) and a rapid normalization of ESR during corticosteroid treatment supports the diagnosis (1 32)

The final diagnosis can be established only by biopsy from an involved artery The histological diagnosis is based on the presence of active inflammatory changes in the vessel wall (2 11 12 16 18) The most constant findings include the following changes intimal thickening possibly with luminal obliteration fragmentation of the internal elastic membrane necrosis and cellular infiltration of the intima the media and the adventitia Giant cells are often seen especially near the fragmented elastic lamina (11 15) The presence of giant cells is not specific for this type of arteritis and cannot be demonstrated in 20–40% of patients with giant-cell arteritis (5 12 28) The arteritis is confined to the large and medium sized arteries especially to the arch of the aorta and its major branches (9 11 12 15) However the arteries are only segmentally af



Fig 1 Branch of arteria temporalis with polymorphous granulations and formation of non-specific giant cells in part of the media and intima Lumen partly occluded by a poorly organized thrombus Diagnosis granulomatous giant cell arteritis Hematoxylin-eosin staining ×175

temporal arteritis and Takayasu s disease has been extensively reviewed and examined by Hamrin (4) In his series of polymyalgia arteritica he found two young patients with arteritis of Takayasu s disease No clinical or histological evidence of involvement of the temporal arteries was however found in these patients and it was pointed out that the identity of these arteritic forms has been neither established nor excluded Our patient displayed no clinical signs of Takayasu s disease

A juvenile atypical" form of temporal arteritis has recently been described (7) Histological examination of the temporal arteries showed a non giant cel granulomatous inflammation Although the le s differed sufficiently from the classical form of temporal arteritis the authors consider the lesions to represent a new clinico-pathological entity— juvenile temporal arteritis In the light of our own report it may be considered inadequate to use the name juvenile temporal arteritis for other young patients than those with giant cells in the biopsy

The concomitant occurrence of signs of ankylosing spondylitis in our young female is also of interest An association between these two diseases is not usually expected because ankylosing spondylitis seldom appears after the age of 50 whereas giant cell arteritis is seldom seen at an earlier age Autoimmune mechanisms have been suggested for both giant cell arteritis (2) and ankylosing spondylitis (5) An association between these two diseases may therefore be taken as further indirect evidence that they are caused or perpetuated by abnormal immune mechanisms

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Evaluation of the Sulphapyridine Acetylator Phenotyping Test in Healthy Subjects and in Patients with Cardiac and Renal Diseases

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ABSTRACT The acetylator phenotype of 35 healthy, drug free volunteers and 21 patients with cardiac and/or renal disease has been assessed using oral sulphapyridine Comparative evaluation of a simplified and a more selective method of sulpha pyridine analysis was performed Thirteen of the pa tients were also phenotyped by determination of plas ma isoniazid half life 81% of the patients were slow acetylators compared with only 51% of the volun teers When phenotyping healthy, drug free subjects the analytical procedure, involving a direct estima tion of sulphapyridine in urine with the Bratton Mar shall procedure, was satisfactory On the other hand, in patients receiving concomitant drug therapy the more selective analytical procedure was necessary in order to diminish the risk of methodological inter ference

The polymorphic acetylation of isoniazid (INH) some sulphonamides hydralazine and procaine amide has been established in many investigations (4 6 7 11 16 17) It has also been shown that side effects of these drugs are more frequent in slow ace tylators (1 2 9 10 14 19 20) Phenotyping of pa tients before the beginning of therapy with these drugs would therefore be of clinical value

Most of the available tests for acetylator pheno type determinations have been developed using healthy volunteers There are few such studies in diseased patients on treatment with various drugs Disease as well as concomitant drug therapy would be capable of influencing the pharmacokinetics of the test substance Possible interference in the chemical method used must be considered as well Thus simple urine tests like the sulphapyridine test proposed by Schröder (17) should be evaluated in patients before starting routine determinations of acetylator phenotype Therefore we have performed such a study of the sulphapyridine test in patients with cardiac and renal diseases We have used the sampling schedule described by Schröder (17) and the chemical methods of Schröder and Evans (18) and Hansson and Sandberg (8) The results have been compared with those obtained with the INH test performed in 13 of the patients Healthy volunteers free of drugs served as a control group

MATERIAL AND METHODS

Patients

Acetylation tests were performed in 21 patients 10 males and 11 females aged 29–77 years (mean 55) Twelve pa tients had renal failure to a variable degree with serum creatinine of more than 115 µmol/l In 7 of these patients creatinine clearance was also determined it varied be tween 3 and 75 ml/min (Table I) Liver function assessed from the serum levels of bilirubin alkaline phosphatases ALAT ASAT and LD was normal in all patients Drugs used were digitalis diuretics antihypertensives analge sics sedatives antibiotics corticoids vitamins and ant acids A complete list for each patient is available on request

Acetylator phenotype was determined with sulphapyri dine in all the patients 13 were also phenotyped with INH one week later

Healthy subjects

Thirty five healthy consenting drug free controls 29 males and 6 females 23–47 years old (mean 26) were phenotyped with sulphapyridine Their state of health was determined by history physical examination ECG and routine laboratory tests including Hb ESR serum crea

Table I *Results of acetylator phenotype determination in 21 patients using the sulphapyridine test analysed according to Hansson & Sandberg and the INH test*

Pat no	Sex	S-creatinine (µmol/l)	*Creatinine* clearance (ml/min)	Percentage acetylation of sulphapyridine according to: Hansson & Sandberg	Schroder & Evans	*INH* half life (h)
Slow acetylators with the sulphapyridine test						
1	♀	62		42	–[a]	
2	♀	71		46	38	
3	♀	71		36	33	
4	♀	80		50	45	
5	♂	80		31	28	3 7
6	♀	88		29	30	3.3
7	♂	88		42	52	
8	♂	115		39	–[a]	
9	♂	141	75	51	55	4 7
10	♀	177		30	84[b]	4 0
11	♂	186		28	36	5 1
12	♂	203		42	43	
13	♂	203	23	29	44	3 6
14	♂	628	11	20	–[a]	5 7
15	♀	928	6	29	35	5 8
16	♂	*1 414*	*4*	*17*	*71*[b]	7 9
17	♀	Regular dialysis		34	47	5 8
Rapid acetylators with the sulphapyridine test						
18	♀	97		72	71	1 7
19	♂	159		77	70	
20	♀	186	16	79	84	–[c]
21	♀	1 149	3	90	55[b]	2 2

[a] Obvious methodological interference an adequate result impossible to calculate
[b] The method gives a false classification
[c] Concentration in plasma < 1 µg/ml at all times after dose

urine and serum levels of bilirubin alkaline phosphatases ALAT ASAT and LD

Sulphapyridine test

A single dose of 500 mg sulphapyridine (Septipulmon® tablets supplied by Pharmacia Sweden) was given to the fasting patients and controls after a blank urine sample had been collected No food was allowed until 2 hours later Urine was collected 7–8 hours after drug ingestion (18) In 7 patients with renal failure urine samples were collected at intervals of 0–7 7–8 8–12 12–24 and 24–48 hours after the dose and a venous blood sample was drawn 7 $^1/_2$ hours after the dose Serum and urine were stored at −20°C until analysed

The urine specimens collected before and after the sulphapyridine dose were analysed according to Schroder & Evans (18) a method for direct estimation in urine with the Bratton & Marshall procedure The analysis was repeated one year later on stored urine samples from the controls All the urine and serum samples were at that time also analysed according to the method of Hansson & Sandberg (8) a procedure involving enzymatic hydrolysis extraction with methylisobutylketone and reextraction into hydrochloric acid before the Bratton & Marshall reaction Subjects with a percentage of acetylated sulphapyridine in urine of more than 65 were classified as rapid acetylators according to Schroder (17)

INH test

The fasting patients received INH (Tibinide®) in a dose of approximately 10 mg/kg orally Heparinized venous blood samples were drawn just before the test dose and 3 5 7 9 and 12 hours after The plasma was stored at −20°C until analysed Plasma concentrations of INH were determined spectrophotometrically according to the method of Maher et al (13) The plasma half lives of INH were calculated from the regression lines representing the logarithmic decay of the concentration with time The antimode between rapid and slow acetylators was set to a plasma half-life of 2 1 hours according to Hanngren et al (7)

RESULTS

Healthy subjects

Rapid and slow acetylators were clearly defined by means of the sulphapyridine test (Fig 1) With the Schröder & Evans method there were 18 slow ace-

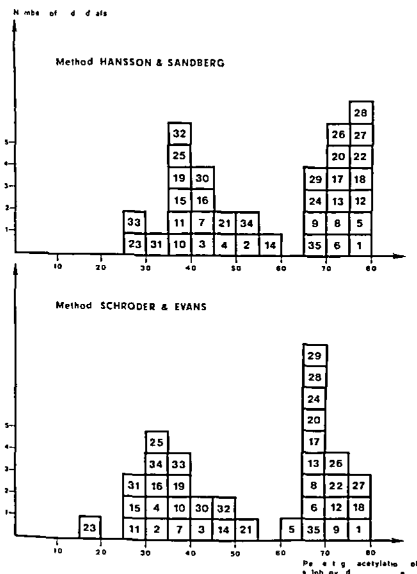


Fig 1 Results of acetylator phenotype determination in healthy volunteers using the sulphapyridine test analysed according to the methods of Hansson & Sandberg and Schroder & Evans Enclosed numerals are codes for healthy subjects

tylators with 36±6% (mean±S D) and 17 rapid acetylators with 72±4% (mean±S D) acetylated sulphapyridine in urine collected 7–8 hours after the test dose

When the urines were reanalysed one year later after storage at −20°C there was agreement regarding the phenotype classification in all but one case This subject had 61 and 70% acetylated sulphapyridine in urine on the two occasions i e border line values With the Hansson & Sandberg method of analysis there were 18 slow acetylators and 17 rapid acetylators with 41±8% (mean±S D) and 73±4% (mean±S D) acetylated sulphapyridine in urine respectively (Fig 1) Here the border line subject had 75% acetylated sulphapyridine in urine Thus 18 (51 5%) of 35 subjects were found to be slow acetylators and the results of phenotype determination with the Schroder & Evans and the Hansson & Sandberg methods of analysis were in good agreement

Patients

In the patient group the sulphapyridine test discriminated slow and rapid acetylators if the urines were analysed according to Hansson and Sandberg (Table I) Seventeen (81%) of 21 patients were slow acetylators

Table II *Acetylation rate of sulphapyridine in urine and serum, using the Hansson & Sandberg method of analysis and INH half life in 7 patients with renal failure*

Pat no	Sex	Creatinine clearance (ml/min)	Percentage acetylation of sulphapyridine: In urine[a] 7–8	8–12	12–24	24–48	In serum[a] 7 5	Acetylator phenotype according to sulphapyridine test	INH half-life (h)
13	♂	23	29	24	39	60	37	Slow	3 6
20	♀	16	79	77	82	84	72	Rapid	–[b]
14	♂	11	20	36	46	54	39	Slow	5 7
15	♀	6	29	33	37	–	44	Slow	5 8
16	♂	4	17	36	30	44	38	Slow	7 9
21	♀	3	90	91	89	94	66	Rapid	2 2
17	♀	Regular dialysis	34	46	45	55	27	Slow	5 8

[a] Hours after dose
[b] Plasma concentrations of <1 μg/ml at all times after dose

For the 7 patients with renal failure the sulphapyridine test gave a clear separation into 2 rapid and 5 slow acetylators in all the urine samples collected 7–8 8–12 12–24 and 24–48 hours after the test dose (Table II) if analysis was performed according to Hansson & Sandberg The two rapid acetylators also had a higher percentage of acetylated sulphapyridine in serum (65 and 72%) than the five slow acetylators (27–44%)

The sulphapyridine test using the Hansson & Sandberg method of analysis was in good agreement with the INH test performed on a separate occasion in 12 of these patients (Tables I and II) The plasma half lives of INH were more than 2 1 hours in all the 10 slow acetylators of sulphapyridine One rapid acetylator had a borderline plasma half life of 2 2 hours It was not possible to determine the plasma half life of INH in the other rapid acetylator due to low serum concentrations

The Schroder & Evans method of analysis gave a different classification in 3 cases and in 3 others it was impossible to calculate an adequate result due to an obvious methodological interference (Table I) In one of the latter cases (no 10) we found a very high value (900 μg/ml) in the hydrolyzed blank urine *which could be traced back to paracetamol* With both methods we found that blank values were higher in patient urine than in control urine Using the Schroder & Evans method blank values in patient urine were 3–100 times higher than in control urine whereas they were only 1–10 times higher with the Hansson & Sandberg method The average blank values with the method of Schroder & Evans were 100% and with the method of Hansson & Sandberg 10% of the concentrations of total and non acetylated sulphapyridine in test urine of patients

DISCUSSION

The frequency of slow acetylators in our patient material with cardiac and renal diseases was 81% In the healthy subjects it was 51% which is within the range reported by Schroder & Evans (18) Admittedly the number of participants is small and the patients and volunteers are not matched regarding sex and age However the same predominance of slow acetylators has also been found when phenotyping patients with spontaneous systemic lupus erythematosus (SLE) (12 16) This difference in the frequency of acetylator phenotype between healthy subjects and patients with various diseases might indicate that slow acetylation implies a greater risk of developing not only the drug induced SLE like syndrome but also various other diseases Alternatively the disease may influence the acetylation capacity

Conventional acetylator phenotyping tests would be irrelevant in patients with renal failure Fine and Sumner (5) found that the conventional sulphadimidine acetylation test in serum and urine incorrectly defined the acetylator phenotype in 50% of patients with severe renal failure on regular dialysis The explanation may be a relatively greater retention of the metabolite which leads to an increased ratio of acetylated drug in the blood and a decreased ratio in urine They concluded that acetylator status in

uremic patients could not be defined unless a very elaborate method was used involving 12 venipunctures collection of urine for 24 hours and calculation of total renal and metabolic clearance of unchanged and acetylated sulphadimidine The results of our investigation are not in accordance with these findings Seven of our patients with renal failure including 4 with a creatinine clearance of less than 10 ml/min exhibited a rather good correlation between the acetylation rate of sulphapyridine in serum and urine A more selective method of analysis and the fact that our patients did not all have severe renal failure would perhaps explain the divergences

Investigation of the acetylation rate in patients is made more difficult by the necessity of continuing concomitant drug therapy These drugs could influence the results of the sulphapyridine test not only through interference with the analytical procedure but also through metabolic interaction The risk of methodological interference depends on the method used for phenotype determination The sulphapyridine test with one urine sample collected 7–8 hours after the dose—performed on well controlled healthy and drug free subjects—seems to give a reliable classification with both methods of chemical analysis In the patient material the methodological situation was quite different The sulphapyridine phenotyping test with the method of analysis according to Schroder and Evans gave inaccurate results in 3 patients and proved impossible to perform adequately in 3 others

The most obvious substances capable of interfering with the method of analysis are compounds with primary aromatic amino groups for instance procaine amide and sulphonamides A second group of interfering substances are those that readily obtain a primary aromatic amino group after hydrolysis such as paracetamol Primary secondary and tertiary amines and phenolic compounds are capable of interfering in other ways in the Bratton & Marshall reaction if present in sufficient quantities Compounds that are dyes in themselves or easily form dyes on hydrolysis may also interfere with the colour of the sulphapyridine–Bratton Marshall complex

The fact that blank values estimated with the Schroder & Evans method were up to 10 times higher than those determined by the Hansson & Sandberg method in our patient material indicates a much higher incidence of interference in the Schroder & Evans procedure This gives a much greater risk of an inaccurate and misleading result Therefore the more selective method of Hansson & Sandberg was investigated With this method there was a good discrimination between rapid and slow acetylators and the results correlated well with those of the INH test

The extraction steps and the enzymatic hydrolysis in the method of Hansson & Sandberg makes this method more elaborate and time-consuming than that of Schröder & Evans In addition the reliability of the Hansson & Sandberg method of analysis is not completely satisfactory because of high blank values in some cases Perhaps a chromatographic method is needed Further studies will show if this is the case

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Spontaneous Systemic Lupus Erythematosus and Acetylator Phenotype

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ABSTRACT Fifteen patients with spontaneous systemic lupus erythematosus (SLE) have been phenotyped by determination of plasma isoniazid (INH) half life Seven patients had signs of renal insufficiency *Of the 15 patients, 13 were slow and only 2 rapid acetylators* No correlation was found between the plasma INH half lives and the renal function Thus, there is the same marked predominance of slow acetylators in patients with spontaneous SLE as in patients with the drug induced SLE like syndrome

Many drugs have been suspected of inducing a syndrome resembling systemic lupus erythematosus (SLE) However this relationship has been verified for only a few Procainamide and hydralazine have been most frequently reported to induce the syndrome but isoniazid (INH) and some anticonvulsants may also precipitate this side-effect (1 15)

Genetic polymorphism of acetylation has been described for both INH hydralazine and procainamide (5 13) and slow acetylation represents an autosomal recessive trait It has also been demonstrated that slow acetylators are more prone to develop side-effects including the drug induced SLE like syndrome after long term therapy with these agents (2 11 13 17 22) It is suggested therefore that acetylation is a detoxification mechanism (4) Slow acetylation may however also reflect a genetically determined predisposition to develop the SLE like syndrome the disease becoming manifest after an interplay of genetic and environmental factors of various intensity (1)

In support of the latter hypothesis Reidenberg and Martin (19) reported a significant predominance of slow acetylators (10 out of 14 subjects) when phenotyping patients with spontaneous non-drug induced SLE syndrome These findings seem to be of great importance when discussing the relation ship between phenotype and drug induced side effects in pharmacokinetic terms As the above patient population was small we have performed a similar study to seek confirmation of these observations

MATERIAL AND METHODS

Fifteen patients 12 females and 3 males 19–65 years old (mean 33) participated in the study None of them had ever received procainamide hydralazine INH anticonvulsants or any other drug suspected of inducing the SLE like syndrome All of the patients gave their informed consent to the investigation

Fourteen patients met at least four of the American Rheumatism Association s criteria indicating SLE (18) One patient met only three of these criteria but had antinuclear antibodies Renal biopsies were performed in 11 patients and glomerular lesions indicating SLE were found in all of them

Seven patients showed signs of renal insufficiency with serum creatinine values of 115 μmol/l or more or endogenous creatinine clearance of less than 70 ml/min at the time of the study The liver function assessed by serum levels of bilirubin alkaline phosphatases ALAT ASAT and LD was normal in all patients

Acetylator phenotype was determined in the following way The fasting patient received INH (Tibinide® Ferrosan) in a dose of approximately 10 mg/kg orally Heparinized blood samples were drawn at 3 5 7 and 9 hours thereafter and analysed according to Maher et al (16) The plasma half-lives of INH and the overall elimination rate constant (λ) were calculated from the regression lines representing the logarithmic decay of the concentration with time The antimode between rapid and slow acetylators was set to a plasma half-life of 2 1 hours according to Hanngren et al (10)

Table I *Renal function and plasma half life of INH in 15 patients with SLE*
S=slow R=rapid acetylator

Pat no	Age (y)	Sex	Serum creatinine (μmol/l)	Creatinine clearance (ml/min)	Plasma INH half life (h)	Acetylator phenotype
1	22	♀	71	121	2 4	S
2	21	♀	80	111	3 6	S
3	29	♀	71	106	4 2	S
4	33	♀	71	103	4 2	S
5	26	♀	62	99	3 6	S
6	19	♀	62	95	4 6	S
7	36	♂	88	80	3 3	S
8	37	♀	71	–	3 1	S
9	38	♀	88	66	3 1	S
10	34	♀	106	36	4 1	S
11	65	♂	141	–	5 9	S
12	21	♀	194	41	1 5	R
13	49	♂	212	36	1 8	R
14	30	♀	354	20	6 4	S
15	30	♀	354	17	4 5	S

All patients received drug treatment during the study including steroids (14 patients) azathioprine (7 patients) and less often cyclophosphamide, antimalarials diuretics and digitalis glycosides

RESULTS

Of the 15 patients 13 were slow and only 2 rapid acetylators (Table I) The mean plasma INH half life in slow acetylators was 4 1 hours (range 2 4–6 4) and in rapid acetylators 1 7 hours (1 5 and 1 8)

Both rapid acetylators showed an impaired renal function with creatinine clearance of 36 and 41 ml/min respectively Of the 13 slow acetylators 5 had renal insufficiency Four of these 5 patients had a mean creatinine clearance of 35 ml/min (range 17–66) and the fifth had a serum creatinine of 141 μmol/l

As shown in Fig 1 there was no correlation ($r=0$ 19) between the renal function assessed by endogenous creatinine clearance and the plasma INH half lives ($n=7$ data are not available on two patients in the slow acetylator group)

DISCUSSION

The results of this study are in close agreement with those of Reidenberg and Martin (19) The marked predominance of slow acetylators in the patients with spontaneous SLE is significantly different from the 50 50 distribution commonly found when investigating both Swedish and North American populations (5 8, 13, 21) However in all of these latter studies the materials consist of healthy volunteers When phenotyping patients of the same ethnic group but with various illnesses such as tuberculosis (10) and acute myocardial infarction (14) the distribution between rapid and slow acetylators was 30 70

The difficulty of studying genetic aspects of the acetylation of drugs in patient materials must be pointed out Most patients received drugs during the study some of which might affect the metabolism of INH although none of them are known to be acetylated Furthermore, no apparent systematic relationship was found between drug therapy and the plasma INH half lives

The plasma half life of INH in patients with renal failure will reflect not only its acetylation but also delayed urinary excretion of INH In 1973 Bowersax et al (3) demonstrated a trend towards prolonged plasma INH half life in patients with renal failure compared with subjects with normal renal function though the difference was not statistically significant In 1972 Jungbluth (12) and in 1973 Reidenberg et al (20) reported plasma INH half lives within the wide range of healthy subjects. Thus acetylation phenotype seems to be a more important determinant of plasma INH half life than renal function In support of this assumption we found no correlation between plasma INH half

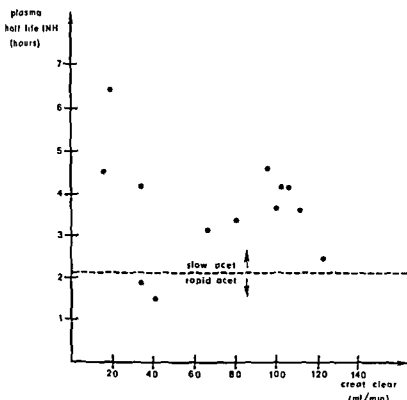


Fig 1 Relationship between renal function and plasma half life of INH in 13 patients with SLE

life and the creatinine clearance value which was available in 13 out of our 15 patients Moreover despite the fact that Reidenberg and Martin (19) excluded all patients with renal failure their results are quite similar to those found in our material which includes patients with renal insufficiency

Antinuclear antibodies have been found in patients with both spontaneous SLE and the drug induced SLE like syndrome (7 9) Slow acetylators are more prone not only to develop the drug induced SLE like syndrome but also spontaneous SLE which would indicate an etiological relationship Slow acetylation might reflect a genetic predisposition to develop both the spontaneous and the drug induced varieties of SLE by causing impaired detoxification ability of both endogenous and exogenous aromatic amine type compounds This would perhaps also increase the propensity to develop other diseases a hypothesis that is supported by the finding of a predominance of slow acetylators compared with healthy subjects not only in patients with SLE but also in patients with other disease

ACKNOWLEDGEMENT

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Cardiac Arrhythmias in Chloral Hydrate Poisoning

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ABSTRACT In three patients admitted to hospital after ingestion of an overdose of chloral hydrate, the ECG showed supraventricular and ventricular tachyarrhythmias The possible mechanism for the arrhythmias may be an enhanced automaticity of supraventricular and ventricular pacemaker cells caused by metabolites of chloral hydrate The ventricular arrhythmia responded to i v treatment with lignocaine in one patient, and to phenytoin in another in whom lignocaine failed to restore a normal sinus rhythm

Chloral hydrate which has been used for more than a century (1) is considered to be a relatively non toxic hypnotic drug and fatalities from overdosage are rare (16) The toxic oral dose in adults is approximately 10 g although fatal poisoning has occurred after ingestion of 4 g (15) On the other hand doses up to 25 g during 20 hours have formerly been used in obstetric therapy apparently without adverse effects (6)

The toxic effects of an overdose of chloralhydrate are characterized by respiratory depression and hypotension Hypotension may be caused by depressed contractility of the myocardium Untoward cardiac effects may occur in particular in patients with heart disease (15)

The aim of the present report is to describe three patients without previous heart disease who showed cardiac arrhythmias after an overdose of chloral hydrate To our knowledge only a few cases with this complication have hitherto been reported (3 4 9 10)

PATIENTS AND METHODS

The observations were made on three patients who were admitted to our Intensive Care Unit after an overdose of chloral hydrate All three had taken Ansopal® tablets and no other drugs One tablet of Ansopal contains 0 5 g chloral hydrate as acetylglycineamide-chloral hydrate Acetylglycineamide is said to be pharmacologically inactive (5)

A 12-lead ECG was taken on admission and the recording was then repeated daily or more often when required Heart rhythm was monitored continuously Routine laboratory examinations including serum electrolytes and blood gases were performed

CASE REPORTS

Case 1

A 39 year-old woman was admitted eight hours after ingestion of about 60 tablets of Ansopal Only a small amount of tablet remnants was recovered by gastric lavage On admission she was comatose but responsive to pain The respiratory rate was 16/min and analysis of an arterial blood sample showed pH 7 33 $PaCO_2$ 5 3 kPa (40 mmHg) PaO_2 9 7 kPa (73 mmHg) base excess −5 0 mmol/l Serum electrolytes were normal The rectal temperature was 37 0°C and BP 105/75 mmHg An endotracheal intubation was performed and treatment with forced diuresis was started Frusemide 20 mg i v was given six hours after admission and another dose of 40 mg was given after 13 hours

An ECG on admission showed sinus rhythm with a rate of 120/min Two hours after admission the continuous monitoring revealed occasional supraventricular and frequent ventricular premature beats of varying morphology in salvos (Fig 1) Lignocaine (Xylocain®) 50 mg i v was given as a bolus injection followed by an infusion of 2 mg/min As no effect on the arrhythmia was noted after one hour the infusion was discontinued Phenytoin (Epanutin®) 150 mg i v was then administered at a rate of 25 mg/min When 125 mg had been given the ventricular premature beats decreased in frequency and immediately after the injection the heart rhythm became regular After half an hour an additional dose of 100 mg phenytoin was given and the rhythm remained normal except for short periods of ventricular bigeminy recorded at 7 and 21 hours after admission A 12 lead ECG showed nothing abnormal except for slight flattening of the T waves The patient recovered consciousness 12 hours af

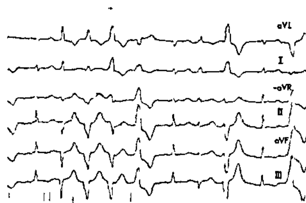


Fig 1 Occasional supraventricular beats and frequent ventricular premature beats after ingestion of about 60 tablets of Ansopal® ten hours earlier (Case 1)

ter admission and was extubated 10 hours later She was discharged on the fourth day of admission

Case 2

A 51 year-old man was admitted about 8 hours after ingestion of approximately 50 tablets of Ansopal (a gastric lavage yielded a large amount of tablet remnants) On admission he was comatose with no reaction to pain The rectal temperature was 36 9°C Respiration was shallow and analysis of an arterial blood sample showed pH 7 32 $PaCO_2$ 6 7 kPa (50 mmHg) PaO_2 7 9 kPa (59 mmHg) standard bicarbonate 23 0 and base excess −1 5 mmol/l Endotracheal intubation was performed and respirator therapy and treatment with forced diuresis were started Frusemide 20 mg i v was given 24 hours after admission

On admission an ECG showed a supraventricular rhythm with a rate of 75/min interpreted as an accelerated AV junctional rhythm since no P waves were identified The heart rate was about 200/min 15 min later The ECG showed broad QRS complexes interrupted by occasional normal complexes and fusion beats (Fig 2) On the diagnosis of ventricular tachycardia lignocaine was given as an i v bolus injection of 70 mg The ECG was recorded continuously during the injection and when 40 mg had been given a supraventricular rhythm with a rate of 150/min appeared The P waves were initially inverted in leads II and III but after a few beats they became upright indicating sinus rhythm Ventricular premature beats with QRS complexes of the same morphology as during the tachycardia appeared during the first minute after conversion followed by regular sinus rhythm Systolic BP decreased to 50 mmHg during the tachycardia but increased immediately after the episode to 80 mmHg Lignocaine was given continuously at a rate of 2 mg/min during the next 30 hours Continuous monitoring revealed no further arrhythmias and the subsequent course was uneventful A 12 lead ECG on the second day of admission was normal and so were the serum enzymes (S ASAT and S ALAT) Respirator therapy was discontinued 30 hours after admission and seven hours later the patient was alert and could be extubated He was discharged on the third day of admission

Case 3

A 21 year-old woman was first admitted to a local hospital one hour after ingestion of about 40 tablets of Ansopal and some alcohol After endotracheal intubation and gastric lavage which yielded a large amount of tablet remnants she was given mannitol and 40 mg frusemide On arrival at the Intensive Care Unit in Lund two hours after ingestion of the tablets she was still comatose but responded to pain The respiratory rate was 14/min and an arterial blood sample taken when an oxygen-enriched air mixture was being administered showed pH 7 35 $PaCO_2$ 4 9 kPa (37 mmHg) and PaO_2 17 9 kPa (134 mmHg) standard bicarbonate 18 5 and base excess −7 0 mmol/l The rectal temperature was 37 0°C The ECG on admission to the Intensive Care Unit showed runs of ventricular tachycardia with QRS complexes of varying morphology (Fig 3) Shortly after recording of this arrhythmia the rhythm spontaneously converted to sinus rhythm with a rate of 150/min No antiarrhythmic therapy was given and the heart rate decreased to 120–100/min Continuous monitoring revealed no arrhythmia during the subsequent course The patient recovered consciousness one hour after admission and was then extubated She was discharged on the second day of admission

DISCUSSION

After absorption a varying amount of chloral hydrate is oxidized to trichloroacetic acid and the rest of the drug is reduced to trichloroethanol (1 8 11) This metabolism is so rapid that no chloral hydrate could be detected in the blood after therapeutic doses in man (8 14) It was detected in blood samples taken after 10 but not after 15 min when large doses were administered orally to dogs (8) The hypnotic effect is largely if not entirely

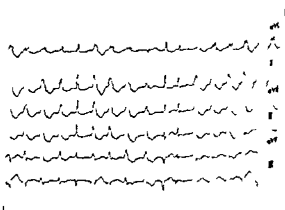


Fig 2 Ventricular tachycardia with occasional supraventricular and fusion beats after ingestion of 50 tablets of Ansopal® eight hours earlier (Case 2)



Fig 3 Runs of ventricular tachycardia with QRS complexes of varying morphology after ingestion of 40 tablets of Ansopal® two hours earlier (Case 3)

due to trichloroethanol while trichloroacetic acid has no such effect (1 8) The plasma half lives of trichloroethanol and trichloroacetic acid have been estimated to be 8 2 and 67 hours respectively (12 13)

In four reports of cardiac arrhythmias after chloral hydrate the ECG showed supraventricular and/or ventricular tachyarrhythmias Auricular fibrillation with a high ventricular rate and ventricular premature beats were observed in a patient 45 min after ingestion of 12 g chloral hydrate (9) Ten hours after ingestion of 18 g ventricular tachycardia was observed in a 66-year old woman This rhythm reverted to sinus rhythm on procainamide therapy but the arrhythmia recurred after three hours with runs of ventricular fibrillation which responded to continued treatment with procainamide (4) Recurrent episodes of ventricular fibrillation premature ventricular beats and bursts of supraventricular and ventricular tachycardia were recorded in a patient with a history of myocardial infarction who had taken 18 g of the drug The arrhythmias were refractory to lignocaine but responded to alprenolol (3) In a 2 year-old child who had ingested 1 5 g chloral hydrate an ECG revealed multifocal premature beats The arrhythmia disappeared spontaneously after about two hours (10)

In the present patients the arrhythmias were of the same type as those described in the above publications in that they all had supraventricular tachycardia and ventricular premature beats in salvos or ventricular tachycardia The mechanism behind these arrhythmias was presumably an enhanced automaticity of supraventricular and ventricular pacemaker cells As the ECGs showed no signs of AV block or intraventricular block a decreased conductivity with re-entrant mechanism seemed to be a less plausible cause The shortening of the refractory period of the myocardium which has been observed after large doses of chloral hydrate (15) could also be of importance for the genesis of the arrhythmias

Since chloral hydrate is metabolized so rapidly and the arrhythmias were observed hours after ingestion of tablets it is most likely that one of the metabolites was the causative factor Trichloroacetic acid has been proposed as being responsible for the tachycardia and vasodilatation following simultaneous administration of chloral hydrate and alcohol (12) and administration of frusemide to patients who had taken chloral hydrate (7) The relapse of the arrhythmias after several hours in some of the previously reported cases and also in the present case 1 may be attributed to the long half life of trichloroacetic acid

In our patient 3 gastric aspiration was performed soon after ingestion of the tablets and the coma was of short duration which indicates absorption of only a moderate amount of the drug yet a period of arrhythmia occurred An interaction of chloral hydrate and alcohol as well as frusemide could have contributed to the origin of the arrhythmia in this case In case 1 ventricular premature beats were observed one hour after administration of frusemide but this arrhythmia also recurred later when no frusemide had been given In case 2 no arrhythmia was recorded when frusemide was given 24 hours after admission

Lignocaine has become the drug of choice in treatment of ventricular tachyarrhythmias In case 2 of the present study the arrhythmia was abolished by lignocaine In two patients unresponsive to lignocaine regular sinus rhythm was restored by alprenolol in one (3) and by phenytoin in the other (our case 1)

The arrhythmias reported in the present study had a benign course but their appearance was that of a potentially malignant ventricular tachyarrhythmia The practical consequence is that monitoring of the heart rhythm is indicated in patients admitted after an overdose of chloral hydrate

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ECG Recording in Emergency Home Visits

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ABSTRACT During the first half of 1974, 756 emergency home calls have been made by 6 doctors in the City of Oslo A portable ECG instrument was available at all visits The visits were unselected as regards complaints ECG was recorded in 7% of all patients and in 19% of all patients over 60 years of age The main indication for recording an ECG was chest pain (nearly 2/3 of all recordings) In only a small number of cases did the recording give a definite result as regards diagnosis or management of the case However, a slight additional benefit was obtained with a majority of the ECG recordings, including what we defined as a psychotherapeutic benefit The results of this study indicate that the value of a portable electrocardiograph is very limited for emergency home calls in a city

A battery-driven portable ECG instrument which is both reliable and reasonably inexpensive has become available This has raised the question whether ECG recording should be encouraged during home visits The present investigation was undertaken to determine the usefulness of this equipment in emergency home calls in the City of Oslo

MATERIAL AND METHODS

Six medical registrars were furnished with a portable electrocardiograph (model FJC 7110 Fukuda Denshi Co Ltd placed at our disposal by Svalland A/S Oslo) when making emergency home calls The calls were arranged by Oslo Emergency Medical Centre The doctors on call visited unselected patients with all types of complaints and ailments When a clinical evaluation had been made an ECG was recorded only in patients for whom this seemed to be indicated

When the ECG recording led to a different diagnosis or another medical management it was classified as of great value When the recording only supported an established medical opinion it was listed as of some value The remaining recordings were defined as of no value We also attempted to classify a psychotherapeutic effect in the same way as we evaluated specific medical conditions

RESULTS

There were no technical difficulties as regards the use of the ECG equipment A 12 lead recording was usually obtained within 12–15 min

ECG was recorded in 51 (7%) of 756 patients and in 19% of patients over 60 years A slight majority of the patients investigated were males (Table I)

Chest pain was the most frequent indication for an ECG recording but acute myocardial infarction was not diagnosed 40% of the ECG recordings were normal (Table II)

Table I *Sex and age distribution of patients investigated with ECG recordings in 756 emergency home calls*

		ECG recorded	
	Total	*n*	% of total
Females	436	24	5 5
Males	320	27	8 4
Total	756	51	6 7
Age (y)			
<15	285	0	
15–30	127	3	2 3
31–60	157	13	8 2
>60	187	35	18 7

Table II *Main indication for ECG recording and ECG diagnosis in 51 patients*

	No of pats
Indication	
Chest pain	32
Congestive heart failure	1
Cardiac arrhythmia	8
Psychological	8
Other	2
Diagnosis	
Normal	21
Ischaemic heart disease	*12*
Ventricular premature beats	8
Atrial/nodal premature beats	1
Atrial/nodal tachycardia	1
Atrial fibrillation/flutter	5
Bundle branch block	4
Left ventricular hypertrophy	2
Healed myocardial infarction	6
Acute myocardial infarction	0

Real benefit (great value) from the ECG record ing was thought to be obtained in only a small number of cases Most recordings simply verified an established diagnosis (Table ' , As far as medi cal management was concerned, the recording was of no consequence in more than 50% of the cases

Of the 14 patients admitted to hospital the ECG was of no value in 10 of some value in 3 and of great value in only one 11 of these 14 patients had chest pain A psychotherapeutic (reassuring) effect was registered in about half of the 51 patients

Table III *Value of ECG recording in 51 patients*

	No of pats		
Value concerning	No value	Some value	Great value
Diagnosis	13	31	7
Management	28	16	7
Decision on admission to hospital	31	16	4
Psychotherapy	24	19	8

DISCUSSION

The present study attempted to determine the value of a portable electrocardiograph in emergency home calls Only in a small number of patients did the recording of an ECG during a home visit lead to a *new diagnosis or different management* of the patient The ECG supported about 60% of the diagnoses However this confirmation was considered to be of limited value and does not suggest that a portable ECG instrument should be available when making emergency home calls in a an urban area The finding of a reassuring effect in many patients does not in our opinion alter this conclusion Our results are no doubt influenced by the fact that in the City of Oslo many patients with acute heart conditions are taken straight to hospital without being seen by a doctor prior to admission It may well be that a similar investigation carried out in a rural district would give different results

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Pattern of Acute Drug Poisoning in Oslo

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ABSTRACT All 1742 admissions occasioned by acute drug poisoning to a medical ward in Oslo during the periods 1958–60, 1963–65, 1966–69 and 1970–73 have been reviewed The number of admissions per annum in these periods was 99, 119, 128 and 144, respectively Because the hospital situation and number of inhabitants in the city remained fairly constant from 1958 to 1973, the increasing figures were assumed to reflect a corresponding increase in the incidence of acute drug poisoning in Oslo The incidence was estimated to be within the range of 1–2 per 1000 inhabitants per annum No female predominance was noted except for the age group under 30 years The occurrence of barbiturate poisonings decreased markedly during the periods studied, whereas those due to non barbiturate hypnotics, tranquillizers and tricyclic antidepressants showed a proportionate increase Slightly less than one fourth of the females and half of the males had taken alcohol More males (43 %) than females (13 %) also were alcoholics A suicidal intention was however found more frequently among females (70 %) than males (43 %) The hospital mortality was approximately 1 % No death was associated with tricyclic antidepressants, which in the last study period had been ingested by approximately 10 % of all patients

Drug poisoning represents a serious and growing problem in most countries Self poisoning has recently been claimed to be the second most common reason for emergency admission to medical wards (13 16 23 31)

The present report deals with the admissions for acute drug poisoning to a medical ward in Oslo and covers a period of 14 years Some of the findings apparently differ from those reported by others

MATERIAL AND METHODS

The study was performed as a retrospective analysis of hospital records and included all cases of acute drug poisoning admitted to Department IX (internal medicine) Ullevål University Hospital Oslo Norway in the periods 1958–60 1963–65 1966–69 and 1970–73 The diagnosis was mainly based on anamnesis and clinical observations Blood concentrations of barbiturates alcohol and salicylate were routinely determined in suspected cases Chronic drug poisoning (e g digitalis) or adverse reactions to drugs were not included in the study No patients admitted for drug poisoning were discharged after preliminary treatment in the emergency room They were always hospitalized for a minimum of 12 hours for observation/treatment

The number and distribution of medical beds in Oslo available to patients with acute drug poisoning remained fairly constant throughout the periods studied Based on information from all medical wards in Oslo the admissions to our department accounted for approximately 20 % of all admissions with this diagnosis in Oslo The population of Oslo in the periods studied was 472 000 486 000 488 000 and 472 000 respectively

The following groups of drugs used for self-poisoning were noted alcohol barbiturates non-barbiturate hypnotics tranquillizers (both major and minor) tricyclic antidepressants salicylates and opiates As to whether suicide was thought to be intended or not the reviewer s judgement was mainly based on an interview by a consulting psychiatrist

The statistical analysis of differences observed was performed with the χ^2 test

RESULTS

The study covered 1742 admissions for acute drug poisoning (821 females 921 males) during a period of 14 years The number of admissions per annum (Fig 1) showed an increase of approximately 40 % from 1958–60 to 1970–73 (60 % for females 32 % for males) However when expressed as a proportion of all admissions the frequency of acute drug poisoning showed little change over the years (range 5 5–6 7 %) The female to male ratio in the four periods studied was 0 73 0 98 0 97 and 0 87 respectively (Fig 1) The duration of hospitalization was reduced from 8 days in 1958–60 to 4 days in

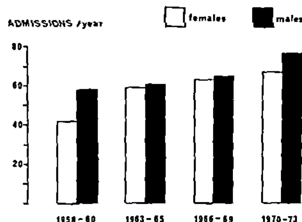


Fig 1 Admissions per year to Department IX Ullevål Hospital Oslo Norway occasioned by acute drug poisoning in the periods studied

1970–73 No difference between sexes was found in this respect

The age and sex distribution is shown in Table I More females than males were under 30 years of age ($p<0.01$) This youngest group tended as a whole to increase and the number of those under 30 was larger in 1970–73 than in any other period ($p<0.05$) The increase was most marked for males

The frequency and duration of unconciousness showed a slight decreasing tendency (Table II) No significant differences were found between females and males

Slightly less than half of the males and one fourth of the females had taken alcohol (Table III) About one half were pure alcohol intoxications whereas the other half had taken alcohol together with a drug Poisoning due to barbiturates showed a marked constant decrease from the first to the last period of the study (Table III) A corresponding increase was noted as regards poisoning due to non barbiturate hypnotics and tranquillizers as

Table I *Age and sex distribution (% of all admissions)*

Period	<30 y ♀	<30 y ♂	30–60 y ♀	30–60 y ♂	>60 y ♀	>60 y ♂
1958–60	30	9	60	71	10	20
1963–65	28	24	58	57	14	19
1966–69	41	26	45	63	14	11
1970–73	48	40	41	47	11	13

Table II *Frequency and duration of unconsciousness on admission*

Period	Frequency (% of all admissions)	Duration (% of those admitted unconscious) <12 h	>24 h
1958–60	28	67	27
1963–65	34	43	36
1966–69	22	61	24
1970–73	22	58	22

well as tricyclic antidepressants The latter type o drugs had been taken in the last period by approxi mately 10% of all cases Slightly less than 5% ha taken salicylates Of the barbiturates allypropym accounted for 60% and pentymal for 35% Amon the non barbiturate hypnotics methaqualone an glutethimide made up 80 and 20% respectively The tranquillizers included both major and mino groups Before 1964 the major group was domi nated by chlorpromazine later by levopromazin and chlorprothixene The minor group was domi nated by meprobamate before 1964 and there after almost solely by benzodiazepine derivates Among the tricyclic antidepressants approximately 50 and 20% were amitriptyline and doxepin re spectively The percentage of patients who ha taken more than one drug tended to increase over the years being in the first period 33% of the fe males and 49% of the males and 49% and 58% respectively in the last period

Gastric aspiration with lavage and i v fluid therapy were used more frequently during the last than the first period of the study ($p<0.01$) wherea the opposite was true for antibiotics ($p<0.01$) (Ta ble IV) No significant differences were found be tween sexes

A suicidal intention was more frequent among females than males ($p<0.01$) More females tha males were also transferred to a psychiatric institu tion ($p<0.01$) whereas more males than females were alcohol ($p<0.01$) and drug abusers ($p<0.01$ (Table V) No changing tendency over the years was noted

Approximately 1% of the patients died in the hospital (Table VI) The drugs involved in cases with lethal outcome were barbiturates salicylate digitoxin promethazine orphenadrine chlorpro thixene levopromazine and meprobamate

Table III *Drugs causing the poisonings (% of all admissions)*

	Alcohol		Barbiturates		Other hypnotics		Tranquillizers		Antidepressants		Salicylates		Opiates	
Period	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
1958–60	20	43	56	47	6	7	16	10	0	0			2	6
1963–65	24	44	37	37	5	7	26	22	5	1			1	3
1966–69	21	51	20	22	18	10	37	21	5	2	6	2	1	2
1970–73	25	49	16	14	27	17	40	30	11	9	4	6	2	5

DISCUSSION

Several authors have suggested that suicide and attempted suicide are two distinct phenomena involving different risk groups and being the expression of different psychological mechanisms (18 33 34 35) Retterstol (26) found that the frequency of suicide in Norway had been fairly constant from 1876 to 1969 and within the range of 7 per 100000 inhabitants which is low compared with many other countries (18) Moreover he found no difference in the incidence of suicide between urban and rural areas this also being in sharp contrast to the situation reported from other countries (18) On the other hand attempted suicide by means of drug poisoning seems to be a problem of urban and dense population areas in our country as well as elsewhere (5 15 18 21 26) stressing the point already mentioned that different risk groups are involved

The frequency of admission for drug poisoning to our department increased 100% from 1945–49 to 1955–57 (15, 21) The increase observed in this study from 1958–60 to 1970–73 was somewhat less about 40% and in line with what has been reported by others (3 7 10 29 30) but far below the increase in incidence reported by most investigators (1 2 11 13 14 16 20 23 31 37) Because the hospital situation admission routine and number of inhabitants in the City of Oslo remained fairly constant from 1958 to 1973 the observed increase in admissions presumably reflects a corresponding increase in the incidence of acute drug poisoning This incidence may be estimated to be within the range of 1–2 per 1000 inhabitants per annum which is lower than the incidence of 2–3 per 1000 inhabitants per annum found in Great Britain (1 2 14 23)

In the present investigation we found that acute drug poisoning accounted for 5–6% of all emergency admissions to our department (90–95% of all admissions to the department are emergency admissions) while 10–30% of all emergency admissions to medical beds in Great Britain concern patients suffering from acute drug poisoning (13 16 23 31)

According to most previous studies drug poisoning is significantly more frequent among females than males The female to male ratios reported have usually been between 1 5 and 2 5 (1 2 7 8 10 13 14 16 17 19 20 24 25 28 29 30 31 37) The present study and previous studies from Oslo (15 21) have failed to disclose any female predominance corresponding well with findings by Swedish investigators (9 11 38)

During the last years of the present study about 40% of the patients were below 30 years of age comparing well with some observations from Denmark and Sweden (7 9 32 38) whereas this age group in Great Britain mostly exceeds 50% (1 2 13 16 17 20) Of 1800 cases of attempted suicide

Table IV *Treatment of acute drug poisoning (% of all admissions)*

Period	Gastric aspiration and lavage	I v maintenance infusion	Antibiotics	Diuretics	Drugs against hypotension	Endotracheal intubation	Assisted ventilation
1958–60	37	30	31		3	8	2
1963–65	53	27	21		13	10	4
1966–69	49	27	8	1	1	5	1
1970–73	67	37	6	4	1	6	2

Endotracheal intubation with mechanical ventilation by pressure-operated respirator

Table V *Psychiatric aspects (% of all admissions)*

Period	Attempted suic de		Alcohol sm		Drug add ction		Repeated admiss on		Transferred to psychiatric care	
	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
1958–60	65	29	15	45	14	25	31	35	9	10
1963–65	65	37	14	44	6	20	23	29	15	1
1966–69	77	54	15	42	12	15	16	19	14	11
1970–73	69	45	9	44	3	14	20	24	18	8

by drug poisoning surveyed between 1964 and 1969 in Milan Italy 90% (76% females) were in the age group 15–25 years (4) In the present study the youngest age group showed the largest numerical increase over the years particularly the males A similar observation was made in Edinburgh Scotland by Aitken et al (1)

The frequency of unconsciousness on admission and its duration tended to decrease over the years and conform well with what have been reported (7 9 16 27) This find ng probably partly reflects changes in the type of drug taken

The occurrence of barbiturate poison ng decreased markedly over the years whereas that of non barb turate hypnotics tranquillizers and tricycl c antidepressants showed a proportionate increase A similar find ng has been reported by others (16 19 ?0 27 30 31 37) A study from the Bergen area in Western Norway showed however that barbiturates in 1969 still accounted for 63% of all drug poisonings in this region (5) Differences in drug prescription trad tions may be respons ble for th s d screpancy The occurrence of salicylate poisoning (5%) was defin tely less than frequently reported (1 13 16 19 20 30 31 37) Opiates taken in overdose actually showed a decreasing tendency over the years wh ch is notable since it does not reflect the increasing narcot c problem in Oslo during the periods studied Alcohol had been ingested alone or together with a drug overdose by slightly less than one fourth of the females and half of the males Patel (?3) found that alcohol abuse preceded drug poison ng in as much as 70% of males and 40% of females A tendency towards po soning with more than one drug seemed to become more pronounced over the years S nce drug po soning s an impulsive action in most instances th s probabl reflects the fact that increasing numbers and varieties of drugs are at hand

Retterstol (26) found that drug po son ng is the most common method of suic de among females in Norway (41%) against only 16% among males In accordance herewith a suicidal intent on in the present study was found more frequently among females than males a fact also reflected by females being transferred to a psych atric ward more often than males More males than females however were chron c alcohol cs and dependent on drugs Several authors have pointed out the marked correlation between alcoholism/drug dependence and suicidal/parasuicidal problems (15 ?? 36) The frequent finding of alcohol abuse concom tant with drug po son ng most probably reflects the same problem

The hospital mortality was about 1% and is a cordant with most other stud es It should be noted that po soning w th tricyclic antidepressants was not fatal in this study whereas h gh mortal ty rates due to these drugs have been reported by others (6)

Table VI *Mortal ty from acute drug poison ng (% of all admiss ons)*

Period	Before reach ng the hosp tal	In the hospital
1958–60	1 0	1 3
1963–65	0 6	? ?
1966–69	0 4	0 6
1970–73	0 5	1 0

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BOOK REVIEW

Tumors of the kidney renal pelvis and ureter Atlas of tumor pathology By James L Bennington and J Bruce Beckwith 353 pages US $6 50 The Armed Forces Institute of Pathology Washington D C 1975

This well known series published by the Armed Forces Institute of Pathology in Washington D C contains a number of excellent monographs of which this is one of the most interesting to the internist Naturally it contains a wealth of information regarding the macroscopical anatomy of tumours in these localizations as well as pictures of light microscopic and electron microscopic preparations of great interest It is really a comprehensive treatise on the morphology of renal tumours but it is much more than that

The text treats embryological epidemiological and clinical questions of great importance The chapter on nephroblastoma (Wilms tumour) is of great biological interest as it stresses the fact that remnants of undifferentiated nephroblastoma occur in multiple sites in the kidneys from tumour patients Careful analysis of the kidneys from newborns dying from other causes show that such embryonic foci occur in 1/100–200 normal kidneys whereas the Wilms tumour is about 100 times more uncommon Neonatal continued differentiation must occur as it does in a somewhat similar paediatric tumour neuroblastoma The connection between lack of development of a certain tissue and malignancy is important for our understanding of cancer The old parallel between foetal cells and carcinoma may have both a morphological and also a metabolic meaning

Excellent data regarding the most important clinical findings that may give a clue to the diagnosis as well as a discussion of metabolic (paraneoplastic) symptoms connected with renal tumours demonstrate interesting chapters in medical oncology

The list of references is very extensive and contains papers from sources outside the American continent The references contain a number of quotations of historical interest and the history of many among these tumours makes fascinating reading e g the chapter on "Grawitz tumour Most important is the fact that the references are remarkably up to date Among important publications you miss the enteroglucagon producing tumour described by Bloom et al from Hammersmith Hospital

This volume will be often consulted not only by pathologists and surgeons but also by all physicians who have an interest in oncological problems

Jan G Waldenström

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Choreoathetosis during Phenytoin Treatment

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ABSTRACT A patient with symptomatic epilepsy receiving only phenytoin developed choreoathetosis and orofacial dyskinesias These movement disorders disappeared when the drug was stopped and reappeared when the patient was challenged Throughout the period of treatment concentrations of phenytoin in serum were consistently low within the therapeutic range Interfering symptoms from the cardiovascular system and the absence of some classic symptoms of phenytoin intoxication (nystagmus and dysarthria) contributed to delay the diagnosis The patient died in hospital and autopsy of the brain showed rather localized encephalomalacies of corpus striatum The pathogenic action of phenytoin and the role of preexisting brain lesions are discussed Phenytoin must be suspected as the cause, when patients on this drug present with uncontrollable epilepsy or neurological or mental deterioration

During nearly 40 years use of phenytoin as an antiepileptic drug a considerable number of well documented side effects have been reported (8) The incidence of untoward reactions is reported to be 15–45% In most instances these reactions are rarely severe or irreversible

Dose-dependent side effects most often produce symptoms from the central nervous system and seem to fall into three groups Firstly the classic symptoms from the cerebellovestibular system consisting of nystagmus ataxia and dysarthria During later years another syndrome so-called chronic phenytoin encephalopathy has been described This includes increased frequency of seizures mental changes certain motor and sensory disturbances together with characteristic EEG changes (8) Finally and more rarely reported are the phenytoin induced involuntary movement disorders These abnormal movements comprise choreoathetosis orofacial dyskinesias (tardive dyskinesias or bucco-linguo-masticatory syndrome) and other movement disorders (e g dystonia hemiballism and myoclonic seizures)

In the available literature at least 17 cases have been reported in whom development of choreoathetosis in various combinations with the above mentioned reactions could be attributed to phenytoin medication (1 4 7 10 11 12 13 14 15 16 17) We report a case of choreoathetosis and orofacial dyskinesias which developed in a patient with symptomatic epilepsy treated solely with phenytoin

CASE REPORT

A 66-year-old woman admitted in Aug 1975 to our department with an acute myocardial infarction cardiac decompensation and bronchopneumonia

In 1974 she had been admitted twice to the department because of grand mal The first seizure was accompanied by a left sided hemiplegia After the second attack she was given phenytoin 400 mg daily In Aug 1975 she was admitted to another hospital because of a new grand mal and cerebral insult with paralysis of the left arm and leg The phenytoin medication was continued in the same dosage and serum values were normal 7 and 11 μg/ml (Department of Clinical Chemistry Glostrup Hospital)

During the first week of the present admission her cardiac complaints were the most prominent problems and she was treated with digoxin diuretics and antibiotics while phenytoin was discontinued After two weeks this medication was resumed with a daily dose of 400 mg (body weight 45 kg) At this time she still complained of dyspnea nausea and vomiting Reduction in digoxin dosage did not relieve the symptoms and determinations of phenytoin concentration in serum were 8 and 10 μg/ml well within the recommended therapeutic range (Medical Laboratory Copenhagen)

At the same time and progressively she was seen to make continuous irregular alternately slow and jerk

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Table I *Variation in phenytoin concentration in serum before during and after challenge of the patient*

The movement disorders were closely related to changes in serum concentration

Date 1975	Dose (mg/d)	Serum concentration of phenytoin (μg/ml)
6/9	400	–
15/9	–	8
24/9	–	10
9/10	Stopped	
17/10	400	0
20/10	–	9
21/10	Stopped	10
22/10	–	7
23/10	–	2
24/10	–	1

involuntary movements of the head accompanied by sighing mouthing lipsmacking and continuous movements of the tongue These writhing movements were also observed in the extremities although to a lesser extent in the left arm and leg due to her slight paresis On standing alone she assumed a lordotic posture falling backwards She could not be induced to walk by herself and with the help of others she produced what looked like an ataxic gait A consultant neurologist described these symptoms as choreoathetoid movements and orofacial dyskinesias of unknown origin There was no known history of neurological disorders in the family Nystagmus dysarthria and gum hyperplasia were not observed

It was only after four weeks on phenytoin that this drug was suspected of causing the disabling symptoms The medication was stopped and during the next week the abnormal movements gradually disappeared She was then quite easily mobilized and could presently walk freely in the ward Only some slight instability of the head was noted

For the future planning of her antiepileptic therapy it was considered important to establish whether phenytoin was the cause of the dyskinesias It was decided to challenge the patient with phenytoin in the same dosage as previously during clinical observation and control of serum values During the next four days she redeveloped the choreoathetoid movements and orofacial dyskinesias The concentration of phenytoin in serum never exceeded 10 μg/ml and the involuntary movements varied with the serum values (Table I)

Phenytoin was then stopped and replaced by phenobarbital 200 mg daily On this drug she became too sedated the drug was stopped and she was discharged without antiepileptic treatment Three days later she was readmitted after another epileptic seizure Carbamazepine was then tried but this drug also produced the involuntary movements even when pimozide was added When these drugs were stopped the symptoms subsided Before other drugs could be tried she suddenly died in the ward

At autopsy the heart was found to be extremely hypertrophied and dilated with severe atherosclerosis of the coronary vessels and mitral incompetence caused by postinfarction fibrosis of a papillary muscle Widespread severe atherosclerosis was found in the whole vascular system of the body

A special autopsy of the brain was performed at the Neuropathological Institute University of Copenhagen On gross examination the brain presented with normal gyri and sulci and meninges without signs of hemorrhage or tumor infiltrations The distribution of grey and white matter was normal and the ventricular system showed normal conditions All the cerebral arteries showed extremely severe atherosclerosis No focal changes particularly in the basal ganglias were observed on macroscopical examination

Numerous small encephalomalacies confined to the corpus striatum on both sides were found histologically These findings were most prominent on the right side in capsula interna where demyelinization was also noted No other focal changes were found the density differentiation and number of cells were normal The number of Purkinje cells in the cerebellum was estimated to be normal

DISCUSSION

We believe that phenytoin in the present case was responsible for the development of the choreoathetoid movements and orofacial dyskinesias because these symptoms disappeared when the drug was stopped and reappeared when it was resumed It is of interest to note that the patient was treated solely with phenytoin for her epilepsy and that the serum values never reached the toxic range even though in terms of body weight she received a large dose In all reported cases in the literature the phenytoin concentration in serum when measured was clearly within the toxic range (>25–30 μg/ml) and the abnormal movements disappeared when the medication was stopped or reduced to therapeutic levels indicating a toxic effect of the drug on the central nervous system (1 11 13) A majority of the reported patients were given combined antiepileptic treatment when presenting with dyskinesias and drug interaction at a neuronal level cannot be ruled out (13)

Common to most of the reported cases is the delay in diagnosing the phenytoin induced movement disorders either due to absence of the more classic symptoms (nystagmus dysarthria ataxia and lethargy) or because an increase in frequency of seizures was thought to indicate undermedication (11 13 16 17) The delay in diagnosing the present patient must similarly be ascribed firstly to ignor

ance of this special phenytoin side-effect but also to the fact that serum values were normal all the time and that nystagmus and dysarthria were absent In addition symptoms from the cardiovascular system obscured the clinical picture

The pathogenesis of phenytoin induced dyskinesias is rather conjectural Analogous drug induced movement disorders caused by long term treatment with phenothiazine or butyrofenon derivates have been extensively reviewed during the last two decades (3, 6) It is postulated that these dyskinesias are a result of neuroleptic induced denervation hypersensitivity of the dopaminergic receptors in the basal ganglias to the normal transmitter substances (9) It has been suggested that older patients and patients with organic brain damage or mental retardation develop involuntary movements more easily and on lower dosages than others (2 5) Some believe that the drug treatment unmasks a preexisting brain lesion which then results in movement disorders

In the present case the rather localized encephalomalacies in corpus striatum could in some way render the patient susceptible to development of movement disorders Acting through the basal ganglias either through a direct toxic effect or by producing denervation hypersensitivity or provoking an imbalance between the various receptor systems phenytoin may have uncovered the movement disorders The observation that carbamazepine chemically a different compound also produced similar symptoms supports this view In this setting our patient may have disclosed what is clinically called senile chorea or arteriosclerotic chorea although it could also be a case of sporadic chorea Huntington

Occurrence of various involuntary movement disorders increased frequency of seizures and mental changes during phenytoin therapy may present as isolated symptoms without the more classic signs of phenytoin intoxication As shown diagnosis may be delayed in patients with normal serum values receiving conventional dosages or when interfering symptoms from coexisting disease are present Phenytoin must be considered as a cause in differential diagnosis of neurological or mental deterioration in any patient receiving this drug

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Thrombocythaemia and Multiple Myeloma

A Report on Two Cases

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ABSTRACT A report is presented on two patients with the very rare combination of thrombocythaemia and multiple myeloma Both patients displayed an increase in monoclonal immunoglobulin in the serum, an increased amount of plasma cells in the bone marrow, and multiple osteolytic lesions in the skeleton, along with a platelet count exceeding 1 mill /mm^3, haemorrhagic diathesis and thromboembolic complications In case 1, both diseases reacted favourably to treatment with melphalan during a 36-month follow up In case 2 the thrombocythaemia had been brought under control with busulphan prior to the diagnosis of myeloma The latter patient died before initiation of treatment of the myeloma The significance of the combination is discussed

As a rule the platelet count in multiple myeloma is normal or slightly diminished prior to treatment with cytotoxic drugs (10) Although thrombocytosis is rare it has been reported in three myeloma cases (14) The highest platelet counts in these patients were 800 000 780 000 and 871 000/mm^3 respectively but there was no mention of haemorrhage or thrombotic complications Moreover three patients with a combination of polycythaemia vera and multiple myeloma have displayed slightly elevated platelet counts (4 9)

Primary or essential thrombocythaemia is one of the myeloproliferative disorders and is characterized by a marked increase in the megakaryocytes of the bone marrow and of the platelets in the peripheral blood along with haemorrhagic and/or thromboembolic complications In general erythropoiesis and myelopoiesis are not affected to the same degree as megakaryocytopoiesis

This report concerns two patients with a combination of essential thrombocythaemia and multiple myeloma

CASE REPORTS

Case 1

Male born in 1895 in good health until 1965 when he was hospitalized due to deep venous thrombosis of the leg He was not anaemic and the platelet count was normal Congestive heart failure was noted and treatment with digitalis was initiated A non toxic goitre was also found and treated surgically in 1966 without per or postoperative complications

In Feb 1973 he developed a second deep venous thrombosis of the leg which was treated with heparin and peroral anticoagulants ESR was 65 mm/h Hypochromic anaemia (erythrocytes 3 8 mill /mm^3 MCH 17) was treated with ferrous sulphate and the Hb concentration rose from 6 7 to 8 9 g/100 ml The patient was discharged with peroral anticoagulants and ferrous sulphate

Despite the iron therapy the Hb did not rise and the patient was readmitted in July 1973 for further examination He complained of fatigue but was otherwise asymptomatic Physical examination revealed pulmonary emphysema and hyperplasia of the prostatic gland the other findings were normal His BP was 160/90 mmHg ESR 37 mm/h Hb 12 8–11 5 g/100 ml Leukocytes were 9 800–12 800/mm^3 with a normal differential count and platelets 980 000–1 420 000/mm^3 Serum iron iron-binding capacity vitamin B_{12} folic acid haptoglobin bilirubin uric acid creatinine and liver enzyme tests were normal Slight proteinuria (0 3 g/l) was observed The blood glucose was slightly elevated glucosuria was not found

In the hospital primary attention was directed towards the prostatic hyperplasia malignancy was suspected Fine needle aspiration biopsy gave a normal cytological finding X-ray surveys of the skeleton showed large osteolytic lesions in the skull in the scapular regions (Fig 1) and in the eighth rib on the left side Bone marrow aspiration showed markedly increased amounts of megakaryocytes but also several groups of immature plasma cells

Table I *Clinical data on two patients with multiple myeloma and primary thrombocythaemia*

	Multiple myeloma			Primary thrombocythaemia		
Pat no	Type of M-component in serum	Plasma cells in bone marrow (%)	Osteolytic lesions	Highest platelet count/mm³	Haemorrhagic diathesis	Thrombo-embolic complications
1	IgA kappa	10 plus lesions with only plasma cells (rib)	Skull shoulders rib	1 420 000	Severe postoperative bleeding	Deep venous thrombosis
2	*IgG kappa*	*30–40*	*Skull femur* Th VI and XI	*1 220 000*	Epistaxis haematemesis subcutaneous bleedings	Deep venous thrombosis pulmonary embolism

The concentration of serum protein was raised to 8 8 g/100 ml with an increase in the γ globulin fraction to 2 25 g/100 ml Determination of the immunoglobulins revealed a rise in IgA to 1 55 g/100 ml immunoelectrophoresis showed this to be monoclonal and of kappa type The concentration of normal IgG was diminished to 0 78 g/100 ml Bence Jones proteinuria was not demonstrable The karyotype was normal 46 XY

Before all this information became available the patient underwent a diagnostic costal resection of the osteolytic lesion The histological examination showed obvious plasma cell infiltration which was consistent with myeloma (Fig 2) The thrombocythaemia led to severe postoperative bleeding with a loss of approximately 2 4 l of blood Furthermore a pleural empyema developed and *a temporary deterioration was apparent in the renal func*tion ESR rose to 137 mm/h The postoperative complications required specific treatment for one month

Treatment with melphalan was given in Oct 1973 in a priming dosage of 10 mg/day for 10 days and 2 mg/day after a two week pause The platelet count decreased gradually and became normal after 11 weeks of treatment The therapy was discontinued after 20 months in conjunction with surgical intervention for a benign mastopathy During melphalan treatment the ESR was completely normalized (137–3 mm/h) The concentration of serum protein became normal the abnormal immunoglobulins rose to normal levels and the paraproteinaemic IgA disappeared The osteolytic bone lesions remained unchanged

No melphalan was given for 7 months By then the platelet count had increased to 660000/mm³ and the therapy was recommenced in a daily maintenance dose of 2 mg The platelet count rapidly became normal This treatment has now been in progress for 15 months The patient is subjectively in good health His renal function is normal and the heart failure is kept under control by a combination of digitalis and diuretics

Case 2

Female born in 1907 whose previous history includes two normal pregnancies In 1949 she underwent cholecystectomy on account of gallstones After the operation her ESR was 8 mm/h In 1958 she suffered a traumatic fracture of the left ankle

The patient was in good health until June 1973 when she experienced her first episodes of epistaxis Her BP (140/85 mmHg) and Hb (13 2–14 0 g/100 ml) were normal No platelet count was performed

The patient was admitted to hospital in Jan 1974 on account of haematemesis Physical examination revealed a normal BP of 150/100 mmHg Subcutaneous bleeding was visible in several places and coagulated blood was present in the nostrils The spleen was moderately enlarged ESR was 58 mm/h Hb 8 1 g/100 ml MCHC 31 2 Leukocytes were 16800/mm³ with a normal differential count and platelets 1220000/mm³ The bleeding time exceeded 15 min X rays of the chest stomach and large bowel were normal, no ulcerations or tumours were detectable The renal function was normal No bone marrow examination was performed

A diagnosis of haemorrhagic thrombocythaemia was made and treatment with busulphan was instituted in a dosage of 4 mg/day for two weeks and then 2 mg/day The anaemia was corrected with blood transfusions The platelet count gradually dropped and became normal 380000/mm³ after 5 weeks of treatment and Hb was 12 6 g/100 ml By now the patient did not display any signs of haemorrhage and the bleeding time was normal

Administration of busulphan 2 mg/day was continued and the patient remained in fairly good health The size of the spleen diminished and after 6 months of therapy it was no longer palpable Hb leukocytes and platelets remained at normal levels

In Aug 1974 the patient suffered a fracture of the right femur and was admitted to a surgical department X-ray of the femur showed large osteolytic lesions around the fracture line similar osteolytic lesions were found in the skull In the vertebral column Th VI and Th XI were collapsed ESR was 92 mm/h Hb 8 9 g/100 ml Erythrocytes were 3 08 mill/mm³ MCH 28 leukocytes 5100/mm³ with a normal differential count and platelets 260000/mm³ Bone marrow examination displayed an abundance of plasma cells amounting to 30–40% of all

...rval between signs of ...mbocythaemia and diagnosis ...myeloma
...o 1973 deep venous thrombosis ...ug 1973 myeloma diagnosed
...ie 1973 epistaxis ...ug 1973 myeloma diagnosed

marrow cells The erythropoiesis was slightly megaloblastic and the myelopoiesis normal The megakaryocytes quantitatively within normal limits were morphologically normal The concentration of serum protein was 8 6 g/100 ml with an increase in the gammaglobulin fraction in electrophoresis to 2 6 g/100 ml This increase was monoclonal and as shown by immunoelectrophoresis of IgG kappa type The concentration of normal immunoglobulins was subnormal The renal function was normal Bence Jones proteinuria was not demonstrable

After two weeks in hospital a deep venous thrombosis was found in the fractured leg and the patient died two days later Pulmonary embolism was suspected but no autopsy was performed

Clinical data on the patients are presented in Table I

DISCUSSION

In both patients the diagnosis of multiple myeloma was clearly demonstrated and was indicated by an increase in plasma cells in the bone marrow multiple osteolytic lesions in the skeleton and a monoclonal increase in serum immunoglobulin Furthermore both patients had haemorrhagic diathesis thrombotic complications and a platelet count in excess of 1 mill /mm³ These findings fulfil the clinical criteria of essential thrombocythaemia (5)

Thrombocytosis may be a significant finding not only in essential thrombocythaemia but also in other myeloproliferative disorders With respect to the cases reported here the normal erythrocyte counts along with the very slightly increased leukocyte counts and the absence of immature myeloid cells in the peripheral blood are not in accordance with the diagnoses of chronic granulocytic leukaemia and primary polycythaemia In patient 1 the karyotype was also normal Although thrombocytosis may also be present in other diseases such as solid tumours ulcerative colitis malignant lymphomas rheumatoid arthritis and amyloidosis (12) it is usually moderate with a platelet count that rarely rises above 800 000/mm³ The patients reported here did not have any symptoms or signs of an underlying disease of this type Concerning amyloidosis it is noteworthy that the monoclonal immunoglobulins were of kappa type which is less often associated with amyloidosis than are the states in which the light chains are of lambda type

In multiple myeloma the platelet count may be normal but thrombocytopenia is common also prior to treatment Elevated platelet counts have been noted in only a few patients (14) but none of them had haemorrhagic diathesis In some other patients a combination of multiple myeloma and polycythaemia vera has been noted Of 13 well documented cases with this combination only three were found to have platelet counts above 400 000/mm³ (1 3 4 6 7 9 13) the highest value was 730 000/mm³ (4)

Both multiple myeloma and thrombocythaemia may develop slowly during a period of several years In the cases under review here the symptoms and signs related to the thrombocythaemia were observed only 6 months (case 1) and 14 months (case 2) prior to detection of the myeloma In case 1 deep venous thrombosis had already oc



Fig 1 Large osteolytic lesions in the left scapula (case 1)

ANNOUNCEMENT

Association of Anaesthetists of Great Britain and Ireland and *Faculty of Anaesthetists of the Royal College of Surgeons of England* invite applications from anaesthetists from any European country with four or more years whole time experience and who expect to be Head of a Department within a few years for *Hoechst Anaesthetic Academic Fellow*. The appointment is for one year and may be in any postgraduate teaching centre in the UK subject to agreement between the Fellow and the teaching centre. The applicant will undertake clinical duties as a senior registrar half time and the remainder in research, teaching or engaged in a clinical area of his or her choice. Salary in the scale of Senior Registrar in the National Health Service.

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Myelofibrosis and Rapid Thrombocytolysis

A Case Report

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ABSTRACT A 69 year-old woman was referred to our department because of moderate anemia and thrombocytopenia On admission the spleen was slightly enlarged On the basis of histological examination of biopsy specimens from spinal processes the diagnosis of myelofibrosis was made The subsequent clinical course was progressively downhill Although splenomegaly was of only moderate degree, severe anemia and thrombocytopenia supervened Platelet mean life span was dramatically shortened (1 8 hours) and platelet production rate considerably increased (about 18×normal) Neither corticosteroid therapy nor splenectomy alleviated the thrombocytopenia Extremely large platelets, with diameters of up to 10 μm, were seen in the peripheral blood The mean platelet diameter and percentage of megathrombocytes reached peak values about 2 weeks after splenectomy It is suggested that the immunologic background of the rapid thrombocytolysis is similar to that which governs platelet destruction in idiopathic thrombocytopenic purpura

The peripheral platelet count in myelofibrosis (MF) is known to be highly variable At the time of diagnosis approximately 1/3 of the patients show subnormal platelet values (20 21) Although the thrombocytopenia is usually of moderate degree it tends to become more pronounced during the course of the disease Platelet survival in MF has been reported to be normal (8) but also slightly or moderately reduced (4 11 19 22) Platelet production in this disease appears to be at least normal and is usually mildly or moderately increased (8 19 22) Marked splenomegaly is mostly present in MF Therefore it seems that gross splenic pooling of platelets may be a major cause of the peripheral thrombocytopenia

The present communication deals with a case of MF with severe thrombocytopenia despite only moderate splenic enlargement Platelet survival was very short and it is suggested that the rapid thrombocytolysis had an immunologic background

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CASE REPORT

A 69-year-old woman had been treated since 1971 because of moderate arterial hypertension initially with propranolol and subsequently with bethanidine and spironolactone Otherwise her past history was non contributory A routine check up in Feb 1974 revealed a mild anemia (Hb 11 1 g/100 ml) and a moderately elevated ESR (41 mm/h) Repeated tests for occult blood in the stools were negative Chest roentgenogram and X ray of the stomach were normal One month later the spleen became palpable and her laboratory findings were Hb 10 0 g/100 ml WBC 4 900/μl platelets 75 000/μl On April 2 1974 she was referred to the Department of Medicine III Sahlgren s Hospital Gothenburg

On admission the patient appeared slightly pale but otherwise in good condition There were no signs of increased bleeding tendency from the skin or mucous membranes No lymphadenopathy or hepatomegaly was present On inspiration the tip of the spleen was palpated just beneath the left costal margin A ^{99m}Tc scintigraphy showed a largest spleen scan area of 80 cm^2 (normal 57±12 (S D)) The laboratory findings were Hb 9 0 g/100 ml RBC 2 6 × 10^6/μl reticulocytes 1 9% WBC 4 200/μl with a percentage of 2 myeloblasts 8 myelocytes 5 metamyelocytes 10 stabs 42 segmented neutrophils 3 eosinophils 2 basophils 2 monocytes and 26 lymphocytes Out of 200 nucleated cells 18 were normoblasts The platelet count was 35 000/μl serum uric acid 7 3 mg/100 ml ESR 87 mm/h serum B_{12} 235 pg/ml and whole blood folic acid 97 ng/ml serum iron 115 μg/100 ml and total iron binding capacity 480 μg/100 ml Direct and indirect Coombs tests were negative COHb was 0 5% The clinical course from the first admission to death is shown in Fig 1

Bone marrow was difficult to aspirate and only sparse material was obtained for examination The differential

CONGRESS ANNOUNCEMENTS

The XXVth Annual Colloquium on Protides of the Biological Fluids will be held in Brugge Belgium May 2-6 1977 The following topics will be discussed Lipoproteins *Cell lines in the study of lymphocyte antigens and receptors* New methods in cell separation

Correspondence Secretariat XXVth Colloquium "Protides of the Biological Fluids" Simon Stevin Instituut Jerusalemstraat 34 B-8000 Brugge Belgium

The First International Symposium on Epidemiological Evaluation of Drugs to evaluate existing data on drug utilization and how epidemiology can be applied to problems of drug effects adverse or beneficial will be held at the Mario Negri Institute Milan, Italy May 2-4 1977 The conference will serve as a background against which a two-day workshop May 4-5 convened by EEG can evaluate whether to proceed with drug surveillance and if so how best to proceed

Organizers The Institute for Pharmacological Research Mario Negri and the Drug Epidemiology Unit of the Boston University

Further information Dr G Tognoni Istituto di Ricerche Farmacologiche Mario Negri Via Eritrea 62 I-20157 Milano Italy

La prochaine session des Journees Internationales de Cardiologie de Paris se tiendra les 16-18 Mai 1977 Le programme comporte une vingtaine de conférences d actualité *et plusieurs tables rondes Le français est la* seule langue utilisée et il n y a pas de traduction simultanée

Pour tous renseignements s adresser au Professeur *J J Welti Hôpital* Fernand *Widal 200 Faubourg Saint* Denis 75010-Paris France

The VIIIth World Congress on the Prevention of Occupational Accidents and Diseases will be held in Bucharest Rumania May 17-21 1977

Organizers the Ministry of Labour of Romania in collaboration with the International Social Security Association (ISSA) and the International Labour Office (ILO) with headquarters at Geneva

Information National Organizing Committee VIIIth World Congress on the Prevention of Occupational Accidents and Diseases Ministry of Labour 1-3 Strada Scaune Bucharest Rumania

An International Symposium on Gut Hormones in Lausanne Switzerland June 18-19 1977 to mark the 75th anniversary of the discovery of the first "hormone" by Bayliss and Starling will review the whole field of gastrointestinal hormones Sponsored by the Widmar Foundation The panel of invited speakers will include Grossman *Arimura Brown Creutzfeldt Fujita Mutt Pearse* Thompson Walsh Said Rehfeld Unger Buchanan Håkanson Gardner Robberecht etc

Information Dr S R Bloom Department of Medicine Hammersmith Hospital du Cane Road London W1? OHS UK

Symposium on Echocardiography with Doppler Applications and New Developments will take place at the Erasmus University Rotterdam the Netherlands June 23-24 1977 Initiated by the Committees on Bio-Engineering and Monitoring of the Seriously ill (comité Recherches Médicales EEC) The purpose of the main program is to present to the clinically oriented participants current uses as well as limitations of echocardiography Lectures (in English) will be given on Doppler and new developments *in two dimensional imaging A limited number of* participants can be accepted Registrations must be made in advance Registration fee D fl 175 –

Further information N Bom Erasmus University *P O Box 1738* Rotterdam The Netherlands

The Sixth International Symposium on Drugs Affecting Lipid Metabolism will be held in Philadelphia Pennsylvania USA Aug 29–Sept 1, 1977

Scientific Secretaries Dr W L Holmes Division of Research Lankenau Hospital Philadelphia and Professor Rodolfo Paoletti Institute of Pharmacology and Pharmacognosy of the University of Milan Italy *Program Chairman* Dr David Kritchevsky The Wistar Institute Philadelphia

Deadline for receipt of abstracts April 15 1977

Further information and forms Dr W L. Holmes Scientific Secretary Symposium on Drugs Affecting *Lipid Metabolism Lankenau Hospital* Philadelphia. Pennsylvania 19151 USA

Second International Congress on Twin Studies will be *held in Washington D C USA Aug 29–Sept 1 1977*

Organizer The International Society for Twin Studies

Further information Dr G Allen Organizing Committee Second International Congress on Twin Studies Clinical Center 2N252 National Institutes of Health Bethesda Maryland 20014 USA

10th International Congress of Chemotherapy will be held in Zurich Switzerland Sept 18-23 1977

Scientific program Symposia and free paper sessions in the field of antimicrobial and anticancer chemotherapy

Correspondence 10th International Congress of Chemotherapy Department of Medicine University of Zurich CH-8091 Zurich Switzerland

Congress on Immunotherapy of Malignant Diseases will be held in Vienna Austria Nov 9-10 1977

Information Dr H Rainer Wiener Medizinische Akademie Alser Strasse 4 A 1090 Wien Austria

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REVIEW ARTICLE

Aminergic Regulation of Thyroid Activity Importance of the Sympathetic Innervation and of the Mast Cells of the Thyroid Gland

Arne Melander

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Some years ago it was discovered that exogenous catecholamines and *5 hydroxytryptamine* (*5 HT* serotonin) are able to induce in vivo secretion of thyroid hormone apparently by direct effects on the thyroid follicle cells (9) The amounts of exogenous amines needed to evoke secretion are large and it is unlikely therefore that the thyroid is continuously stimulated by endogenous amines from the general circulation However it is conceivable that amines located within the thyroid can be released close to the follicle cells and thus affect their activity This fostered a series of morphologic and functional investigations concerning the following questions What amines and what amine containing cell systems are present in the thyroid? How are they distributed within the gland and what is their relation to the follicle cells? Do these amines influence thyroid activity and if so what is the physiological and clinical significance of this influence?

At least three different amine containing cell systems are present within the thyroid namely sympathetic adrenergic nerves mast cells and parafollicular (or C) cells (12) The present essay deals with the two former systems i e the sympathetic innervation and the mast cells and with their possible role in thyroid physiology pathophysiology and pharmacology

SYMPATHETIC REGULATION OF THYROID ACTIVITY

The possibility that the sympathetic nervous system participates in the regulation of thyroid activity has been a subject of much clinical and experimental interest Shortly after hyperthyroidism had been described by Graves and von Basedow it was suggested that the disease might be caused by overactivity in the cervical sympathetic nerves (4) The discovery of adrenaline was soon followed by the observation that this catecholamine can produce several of the phenomena characterizing hyperthyroidism (4) Pathological changes in cervical sympathetic structures were observed in hyperthyroid patients and efforts were made to cure the disease by cervical sympathectomy (4) This therapeutic approach was soon abandoned but current remedies for hyperthyroidism include anti andrenergic drugs both in the preparation of hyperthyroid patients for thyroidectomy and in the management of thyrotoxic crises (4 5 23 25) However this is based on the assumption that anti adrenergic drugs interfere with the effect of thyroid hormone not with its secretion or production Furthermore it seems well established today that genetic immunologic mechanisms play an important role in the development of hyperthyroidism Nevertheless if the sympathetic nervous system does participate in the physiologic control of thyroid function a sudden increase in sympathetic nervous activity might serve to initiate or accentuate hyperthyroidism while immunologic mechanisms per

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This essay was awarded the Sir Charles Harington Prize of the European Thyroid Association 1976

Upon editorial request references have been largely limited to reviews or similar papers

petuate the disease Thus it is important from both clinical and theoretical standpoints to ascertain whether or not the sympathetic nervous system can and does exert an influence on thyroid activity

Experimentally several earlier studies infer that thyroid function can be enhanced under conditions with an increased sympathetic nervous activity but many investigations have given the opposite impression (4 12 22 25) Similarly numerous studies on the effects of exogenous catecholamines on thyroid function have yielded equivocal results Indeed both stimulatory and inhibitory effects have been recorded and it has also been argued that the influence of catecholamines on the thyroid is restricted to effects on the glandular blood flow (4 12 22)

Recent investigations have shown that there are several possible explanations for the divergent opinions concerning the impact of the sympathetic adrenergic system on thyroid function For one thing it has been observed that the frequency and distribution of sympathetic nerve fibres particularly of those reaching the follicles show a great interspecies variation Thus interfollicular sympathetic nerve endings are numerous in the thyroid of the adult hamster sheep mouse and man but very sparse in the thyroid of the adult rat dog and pig (12 19) Moreover there is a pronounced decline in the number of interfollicular symphatetic nerve fibres with increasing age (12 19)

Another important fact is that there are complex interactions between catecholamines TSH and thyroid hormone Thus it is unquestionable that catecholamines can induce secretion of thyroid hormone (see below) but this effect need not be disclosed by measurements of the plasma levels of thyroid hormone as catecholamines also can enchance the peripheral turnover of thyroid hormone (3 6) In addition catecholamines may affect the secretion of TSH and catecholamine induced changes in thyroid blood flow may alter the distribution of TSH to—as well as the outflow of thyroid hormone from—the gland (12 22) Even more important is the fact that exogenous catecholamines and TSH can exert either additive or mutually antagonistic effects on thyroid hormone secretion depending on the timing of their administration relative to one another and on the prevailing state of thyroid activity (15) It follows that valid information on the possible influence of the sympathetic nervous system on thyroid activity can be obtained only after the thyroidal sympathetic innervation has been examined in the species under study In addition the degree of exposure to TSH must be known and controlled Furthermore as measurements of the blood levels of thyroid hormone may yield insufficient information direct morphologic examination of the secretory process is advisable Finally clinically relevant information on sympathetic thyroid relations can be obtained only by studies in man

The presence of catecholamines can be revealed by fluorescence histochemistry of tissue specimens treated with formaldehyde vapour Using this technique it has been established that there are numerous adrenergic nerve terminals in normal thyroid tissue from man mouse hamster and sheep (12) Such terminals are present not only in a network around vessels but also between and around follicles (12) As they disappear following surgical or chemical sympathectomy they are most certainly sympathetic postganglionic noradrenaline containing nerve terminals (12) In order to assess more closely the morphologic relation between the sympathetic nerve terminals and the follicle cells thyroids from normal mice injected with ^{3}H noradrenaline and normal human thyroid specimens incubated with ^{3}H noradrenaline have been examined by electron microscopic autoradiography (12) The labelled catecholamine is taken up by adrenergic but not by other nerve terminals and since the radiation causes precipitation of silver grains in the autoradiographic emulsion adrenergic nerve terminals can be identified With this technique it has been found that the interfollicular sympathetic nerve terminals have a very close relation to the follicle cells as well as to capillaries and arteriolar walls Accordingly there is a morphologic basis for a direct non vascular influence of sympathetic stimuli on the follicle cell of the human as well as of the murine thyroid (12) In addition sympathetic stimuli may influence thyroid function through direct effects on the microcirculation of the gland (12)

There is accumulating evidence that sympathetic stimuli indeed influence thyroid activity by effects within the gland In mice whose TSH secretion has been eliminated in order to avoid indirect effects and interactions as described above electric sympathetic stimulation or drug induced release of noradrenaline or administration of noradrenaline or other catecholamines all induce secretion of

thyroid hormone as reflected by both electron and light microscopic signs of endocytosis of thyroglobulin migration of lysosomes toward the engulfed thyroglobulin and by the release of thyroidal radioiodine into the blood (9 12 13) In addition the secretory response to unilateral sympathetic nerve stimulation is restricted to the thyroid portions that are innervated by the stimulated nerve (13) Hence it is most probable that the effect is evoked by a direct action of noradrenaline released from nerve endings within the gland (13) Moreover the effect is probably induced by the amine as such because the response to exogenous amines is augmented following drug induced inhibition of monamine oxidase and because the catecholamine precursor DOPA exerts a thyroid stimulating effect only after its decarboxylation to dopamine (12)

As already stated there is a morphologic basis for a sympathetic influence not only on thyroid follicle cells but also on thyroidal microcirculation and it is well known that catecholamines affect thyroid blood flow (see above) However since catecholamines stimulate thyroid hormone secretion in the absence of TSH and since they do so irrespective of whether their action is to dilate or constrict thyroid vessels the sympathetic adrenergic activation of thyroid hormone secretion probably results from an action directly on thyroid follicle cells rather than from an influence on thyroidal microcirculation (12) Moreover preliminary studies on normal human thyroid tissue suggest that noradrenaline can induce secretion of thyroid hormone in vitro as reflected by colloid droplet formation and migration of lysosomes Finally there is unequivocal evidence that catecholamines can exert stimulatory effects directly on thyroid follicle cells catecholamines have been shown to enhance the incorporation of iodine and the synthesis of thyroid hormone in isolated calf thyroid cells (7 8 18)

Both TSH and catecholamines stimulate adenylate cyclase in the follicle cell and the stimulatory effect of each on hormone secretion is mimicked by the dibutyryl derivate of cyclic AMP (7 8 12) Moreover both in vivo and in vitro studies indicate that the thyroid stimulating effect of catecholamines is abolished by drugs that block adrenergic receptors (7 8 12) The effect of TSH on the other hand is not blocked by these agents but is inhibited by polyphloretin phosphate a drug that does not diminish the effect of catecholamines (12 17) Finally the in vivo effect of dibutyryl AMP is blocked neither by adrenergic receptor blockers nor by polyphloretin phosphate (12 17) From these findings it seems logical to assume that TSH and catecholamines interact with different receptors on the follicle cell membrane but that both then activate adenylate cyclase and increase the formation of cyclic AMP which in turn mediates their common action on the endocytosis and release of thyroid hormone (12)

The fact that there is a morphologic basis for and experimental evidence in favour of a sympathetic influence on thyroid activity need not signify that this mechanism is of major physiologic or clinical importance To penetrate this question studies have been carried out to determine to what extent the secretion of thyroid hormone is altered in euthyroid organisms exposed to withdrawal or increase of sympathetic nervous activity In mice with an intact TSH secretion sympathectomy evokes a moderate and short lasting reduction in thyroid hormone secretion (11 12) In physically healthy men treated with the sympathomimetic drug amphetamine because of mental disease a moderate rise in the blood level of both T_3 and T_4 parallels the increase of the amphetamine level in blood (10) Concomitantly there is no increase but rather a reduction of the plasma TSH level (10) From these findings it seems reasonable to assume that amphetamine promotes a moderate increase in the secretion of thyroid hormone via release of noradrenaline from intrathyroidal nerve endings (10)

In conclusion it appears that in man as well as in certain other mammalian species there is morphologic and functional evidence for a direct influence of sympathetic stimuli on thyroid activity The tonic sympathetic influence may be of minor importance but the existence of a direct pathway between the sympathetic nervous system and the thyroid gland constitutes a means for rapid adaptations of thyroid hormone secretion to certain stimuli It is possible that an abrupt increase in sympathetic activity may help to initiate and/or accentuate hyperthyroidism

AMINE-CONTAINING THYROID MAST CELLS AND THYROID ACTIVITY

Apart from noradrenaline present in sympathetic nerves amines are found within thyroid mast cells

Histamine a heterocyclic diamine seems to be present in mast cells of all mammalian species including man (21 24) In addition to histamine mast cells in some—but not all—mammalian species contain an aromatic monoamine which is either 5 HT or dopamine 5-HT is found in mast cells of rodents such as rats and mice (1 12 24) while dopamine is present in mast cells of ruminants such as cattle sheep and goats (12 18)

Thyroid mast cells are usually located both in perifollicular and in perivascular spaces Their number is particularly large in the rat whereas the mouse thyroid normally contains very few mast cells (1 2 12 14) This is interesting in view of the fact that the plasma TSH level is high in the normal rat and low in the normal mouse (9) This inference of a relation between the plasma TSH level and the number of mast cells within the thyroid gains support from investigations showing that in both mice and rats long term enhancement of TSH secretion is accompanied by an increased number of mast cells within but apparently not outside the thyroid (12) In addition a recent study suggests that the number of mast cells can be increased also by another thyroid stimulating agent namely the long acting thyroid stimulator (LATS) occurring in serum of some patients with hyperthyroidism (12 16) Accordingly it appears that TSH and perhaps other thyroid stimulators as well promote the formation of mast cells within but not outside the thyroid and it is thus possible that thyroid mast cells are integrated in the regulation of thyroid function (12)

The precise role of thyroid mast cells remains to be elucidated but several observations infer that they are involved in the control of both thyroid blood flow and the secretion and synthesis of thyroid hormone In rats TSH not only promotes the formation of thyroid mast cells but it has also been found to stimulate the release of 5 HT and histamine from such cells most probably by a direct effect on these cells (1 2 9 12) TSH is known to stimulate thyroid blood flow (22) and the thyroidal uptake of ^{86}Rb has been used to study this phenomenon Since TSH mobilizes 5-HT from thyroid mast cells and enhances the thyroidal uptake of ^{86}Rb it has been postulated that the increase in thyroid blood flow evoked by THS is mediated by 5 HT released from thyroid mast cells by TSH (1 2) However since also histamine can be released by TSH it is an open question which of the two amines—if any—is responsible for the effect. Moreover because 5 HT can exert a direct stimulatory action on thyroid follicle cells (12 18) the influence of intrathyroidally liberated amines need not be restricted to effects on blood flow

In order to evaluate how thyroid function can be affected by mast cell amines experimental use has been made of compound 48/80 (a polymer of paramethoxyphenylethylmethylamine and formaldehyde) which causes a rapid and pronounced release of amines from mast cells In rats pretreated with large doses of thyroxine to eliminate the influence of endogenous TSH a single injection of compound 48/80 induces a marked release of 5-HT and histamine from mast cells both within and outside the thyroid Less than 10 min after treatment the depletion of granules 5-HT and histamine is so pronounced that the thyroid mast cells are difficult to visualize by fluorescence microscopy (12, 20) Parallel measurements of the thyroid histamine concentration show an 85 % reduction (12 20)

This amine release is accompanied by enhancements of 1) the thyroid uptake of ^{86}Rb 2) the endocytosis and release of thyroid hormone 3) the thyroid incorporation of radioiodine (12, 20) In accordance with previous investigations it is assumed that the increased uptake of ^{86}Rb—being found together with an increase in thyroid wet weight—indicates that thyroid blood flow has been increased probably in association with an increase of capillary permeability As the effect can be mimicked by exogenous 5-HT or histamine it seems likely that the stimulatory effect of compound 48/80 on the thyroidal uptake of ^{86}Rb in the rat is caused by 5-HT or histamine released from mast cells within the gland In other words 5-HT and histamine released from mast cells within the thyroid can increase the blood flow and capillary permeability in the gland As TSH stimulates the release of both 5-HT and histamine from thyroid mast cells it seems probable that the TSH induced increase in thyroid blood flow and capillary permeability can be mediated by 5-HT and/or histamine released from thyroid mast cells by TSH (12)

As stated compound 48/80 has been found to induce secretion of thyroid hormone in the rat and the effect can be mimicked by exogenous 5-HT but not by histamine Accordingly it appears that 5-HT liberated from thyroid mast cells can stimulate the secretion of thyroid hormone in the rat and it

seems probable that this is a consequence of a direct effect on the follicle cells (12)

The stimulatory effect of 5 HT on thyroid hormone secretion is prevented by α and unaffected by β adrenoreceptor antagonists (12) This could signify that the receptors mediating the effect of 5 HT are identical with the α adrenergic receptors that presumably mediate the stimulation of thyroid hormone secretion by endogenous and exogenous catecholamines (12)

Some recent findings suggest that mast cell amines may influence not only the secretion but also the synthesis of thyroid hormone In cell suspensions of calf thyroid tissue numerous mast cells are recovered together with the follicle cells (18) Instead of 5 HT bovine and other ruminant mast cells contain dopamine (12 18 see above) Dopamine and histamine are retained within the mast cells during the preparation of thyroid cell suspensions and both amines are released from the mast cells in the suspension when compound 48/80 is added (12 18)

Parallel with this amine release there is an increase in the incorporation of iodine into thyroid protein (12 18) Such an effect is recorded also after incubation of cell suspensions with dopamine as well as with noradrenaline adrenaline and 5 HT (7 8 12 18) The incorporation of iodine includes formation of thyroid hormone and the effect of each of the aromatic monoamines is prevented by α but not by β adrenoreceptor antagonists (7 8 12 18) Thus it seems possible that dopamine liberated from thyroid mast cells can exert a direct stimulatory effect on the synthesis of thyroid hormone in cattle and that the effect is mediated by α adrenergic receptors in the follicle cells (12 18) Apparently the same receptors may be involved in the response to other aromatic monoamines and they may be identical with those mediating the stimulation of thyroid hormone secretion by catecholamines and by 5 HT (see above)

In contrast to the aromatic monoamines histamine in doses equimolar to those of the monoamines does not stimulate thyroid hormone synthesis in cell suspensions (12 18) Similarly histamine does not seem to induce secretion of thyroid hormone (12) Thus it appears that the direct influence of histamine released from thyroid mast cells may be restricted to effects on thyroid vessels However this does not exclude the possibility that histamine directly influences the in vivo synthesis of thyroid hormone its effect on blood flow and capillarly permeability may facilitate the uptake of iodine and other substrates (12 18)

To summarize TSH appears to promote not only the formation of thyroid mast cells but also the release of histamine and—when present—5 HT or dopamine from these cells The mobilized amines may function as mediators of the TSH induced increment in thyroid blood flow and capillary permeability and this in turn may facilitate the uptake of substrates for the synthesis of thyroid hormone 5 HT and dopamine released from thyroid mast cells may exert a direct stimulatory effect on the secretion and synthesis of thyroid hormone in addition to their possible effect on thyroid blood flow This direct effect may be mediated by the same (α adrenergic) receptors in the follicle cells as are involved in the sympathetic adrenergic stimulation of thyroid hormone secretion At least one thyroid stimulating immunoglobulin LATS appears to promote the formation of amine-containing thyroid mast cells Hence it seems possible that thyroid mast cells via their amines may play a role in the development of hyperthyroidism

ACKNOWLEDGEMENT

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Comparison between Serum Thyroxine and Triiodothyronine Estimation and the TRH Test in the Routine Diagnosis of Hyperthyroidism

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ABSTRACT Serum T_3 and T_4 levels have been determined by a radioimmunoassay technique and the TRH test has been performed in 50 patients in whom hyperthyroidism could not be ruled out by the first clinical examination alone Each patient was then further evaluated in order to establish the state of the thyroid function The extent to which the determination of T_3 or T_4 could replace the TRH test in the routine diagnosis of hyperthyroidism was evaluated The results showed that 26 of the 50 patients had normal thyroid function and 24 had hyperthyroidism No patient in the normal group and all but one in the hyperthyroid group had T_3 levels above the upper normal limit (2 S D) Two of the patients in the normal group and 19 in the hyperthyroid group had T_4 levels above the upper normal limit (2 S D) Twenty of the patients in the normal group showed a normal TSH response to TRH (increment >3 0 μU/ml) the remaining 6 showed an impaired or absent response Twenty of the hyperthyroid patients had no response and four had a slightly positive response to TRH No hyperthyroid patient had a TSH response exceeding 3 0 μU/ml It is concluded that the determination of T_3 is superior to both the determination of T_4 and the TRH test for the laboratory discrimination between eu and hyperthyroidism

The recent development of radioimmunoassay in the determination of serum thyroxine (T_4) and serum triiodothyronine (T_3) has improved the accuracy with which hormone levels can be estimated in normal and pathological conditions (8 14) In recent years the TRH test has been developed and has proved to be of value in the diagnosis of hyper and hypothyroidism (6) The TRH test is based upon the observation that TSH increases after administration of TRH in euthyroid and hypothyroid subjects but is unaltered in untreated hyperthyroidism

The radioimmunoassay procedure for T_4 and T_3 determination in human serum is rapid and simple The hormones can be assayed simultaneously using 25 μl serum Approximately 150 human sera can be analyzed in any one day by one technical assistant (9) The TRH test is more time-consuming in terms of performance as well as laboratory procedure Serum samples must be collected during 1–2 hours after administration of TRH and each sample must be analyzed with respect to TSH content Each TRH test requires 4–5 samples

The purpose of the present investigation was to elucidate the extent to which the radioimmunoassay of T_4 and T_3 could replace the TRH test in the routine diagnosis of hyperthyroidism

MATERIAL AND METHODS

Patients The levels of T_4 and T_3 in peripheral venous blood were assayed and the TRH test was performed in 50 patients who were referred to doctors in the medical clinics of our hospitals In these patients the diagnosis of hyperthyroidism could not be rejected by the first clinical examination alone The suspicion of hyperthyroidism generally arose from the presence of one or several of the usual symptoms or signs e g increased nervousness insomnia sweating palpitation weight loss or dyspnea in the case of symptoms and e g sinus tachycardia atrial fibrillation tremor or laboratory findings such as slight hypercalcemia or elevated alkaline phosphatase levels in the case of signs

Each patient was then evaluated clinically by one of the authors and further functional tests were conducted to establish the state of the thyroid function The additional tests included determination of the hormone levels in

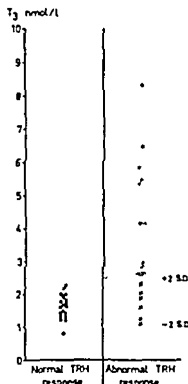


Fig 1 Serum levels of T_3 in patients with normal (TSH increment >3 0 μU/ml) and abnormal (TSH increment <3 0 μU/ml) response to TRH ● = normal TRH response euthyroid × = abnormal TRH response euthyroid O = abnormal TRH response hyperthyroid

serum by competitive protein-binding techniques and estimation of the hormone binding proteins as well as estimation of the thyroidal radioiodine uptake and the T_3 or T_4 suppression test The suppression test was however not performed in patients in whom the diagnosis of eu or hyperthyroidism was already quite clear from symptoms signs and other thyroid laboratory parameters The patients were divided into one normal and one hyperthyroid group

The results from all patients over the age of 60 years were also studied separately to investigate the recently described effect of the age related decline in hormone levels and response to TRH (5 11 13)

Thyroxine and triiodothyronine were determined by a recently described radioimmunoassay technique (9) The normal level for T_4 is 89±35 and for T_3 1 77±0 68 nmol/l serum (mean±2 S D) for subjects between 15 and 60 years of age

TRH test was performed on an out patient basis Synthetic TRH (Hoffman–La Roche Basel) 200 μg i v was rapidly injected The levels of TSH using a commercially available kit (Phadebas TSH test Pharmacia Uppsala Sweden) were determined in serum samples taken before 20 60 and usually 120 min after the TRH injection The results were interpreted in accordance with our standard procedure i e an increment of more than 3 0 μU/ml was regarded as a normal response for all age groups The normal serum TSH level for men was <7 2 and for women <5 5 μU/ml

Additional tests

The serum hormone levels were estimated by the use of the commercial kits Thyropac 3 and Thyropac-4 (Radiochemical Centre Amersham England) The normal value for Thyropac 3 is for men 90–115% and for women 94–119% and for Thyropac-4 60–170 μmol/l

The thyroidal radioiodine uptake was estimated according to the standard procedure The T_3 suppression test was performed by means of administration of 20 μg T_3×5 for 6 days and estimation of the 24 hour radioiodine uptake before and after the administration A fall in the 24-hour uptake of more than 50% of the initial value was regarded as normal The T_4 suppression test was performed by means of oral administration of 3 0 mg T_4 as a single dose (15) The 24 hour radioiodine uptake was estimated before and 7 days after the drug administration A fall of 50% or more was regarded as normal

The additional tests were analyzed routinely by the Isotope and Chemical Departments of our hospitals

RESULTS

In the total material of 50 patients 26 had normal thyroid function and 24 had hyperthyroidism when assessed from the complete clinical and laboratory evaluation The initial symptoms and signs in all patients classified as normals were found to be due to non thyroidal disorders and were normalized by appropriate treatment All patients classified as hyperthyroid responded adequately to treatment with radioiodine surgery or antithyroid drugs

T_3 levels The results are illustrated in Fig 1 No patient in the normal group had a T_3 level above the upper normal limit of 2 S D One of the 24 patients in the hyperthyroid group a 44 year old woman had a normal level of T_3 She had a solitary toxic adenoma no response to TRH and an abnormal T_4 suppression test with a fall in radioiodine uptake from 46 to 38% Her T_4 level was 119 7 nmol/l The clinical symptoms varied from time to time between the presence and absence of signs of hyperthyroidism The symptoms disappeared after treatment Thus none of the normals and one of the hyperthyroid patients showed an overlap

T_4 levels As shown in Fig 2 two of the 26 patients in the normal group had a T_4 level above the upper normal limit of 2 S D They both had a normal T_3 level and a normal TRH test One of these two a 63 year-old housewife with psychiatric problems and on tranquillizers had a normal T_3 level (1 98 nmol/l) an elevated TBG level and a

Table I *Serum levels of T_3 and T_4 and response to TRH in 6 normal patients with subnormal incremental response to TRH and in 4 hyperthyroid patients with response to TRH*

Pat no	Age (y)	Sex	T_3 (nmol/l)	T_4 (nmol/l)	TSH response to TRH (µU/ml) Baseline	Maximum	Increment
Normal group							
1	31	♂	1 87	67	2 5	5 4	2 9
2	32	♂	1 23	76	1 7	3 5	1 8
3	50	♂	2 32	106	<1 5	<1 5	0
4	55	♀	1 58	77	1 5	4 4	2 9
5	62	♀	1 77	77	<1 5	3 0	>1 5
6	64	♀	2 04	99	4 2	6 7	2 5
Hyperthyroid group							
1	25	♀	7 23	189	1 7	4 4	2 7
2	44	♂	2 92	136	1 8	3 9	2 1
3	63	♀	5 25	189	1 7	2 5	0 8
4	67	♂	3 49	138	3 3	4 3	1 0
Normal values (2 S D)			1 09–2 45	54–124			>3 0

normal T_3 suppression test (24 hour uptake decreased from 33 to 10% after T_3 administration) The other a 73 year-old widow with diabetes mellitus and decrease in weight had a normal T_3 level (1 61 nmol/l) and normal values in other thyroid tests Her weight normalized upon treatment of the diabetes

Five of the 24 hyperthyroid patients had normal T_4 levels One of these also had a T_3 level within the normal range and is described above The remaining four had only slightly elevated T_3 levels (2 62 2 85 2 95 and 3 24 nmol/l respectively) All four had a negative response to TRH and abnormal suppression tests All had clinical signs and symptoms of hyperthyroidism which were normalized by treatment Three of these four patients had toxic multinodular goiters and one had a solitary toxic adenoma as judged from the scintiscans

Thus two (8%) of the normals had T_4 levels within the hyperthyroid range In one of them this could be explained by elevated TBG levels No explanation was found in the other Five (21%) of the hyperthyroid patients had normal T_4 levels All had only slightly elevated T_3 levels All had toxic nodular goiters or solitary toxic adenoma

TRH response Twenty of the 26 normal subjects showed a normal TSH response to TRH and 6 an impaired or no response as indicated in Table I which also shows that all 6 had normal levels of T_3 and T_4 Patient 1 a 31 year-old man had been nervous for many years His additional thyroid tests were normal The 24 hour radioiodine uptake decreased from 29 to 8% during the T_4 suppression test Patient 2 a 32 year old man had diabetes mellitus He too had been nervous for many years All additional tests were normal suppression test

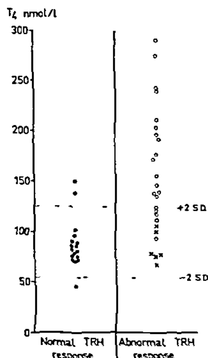


Fig 2 Serum levels of T_4 in patients with normal and abnormal response to TRH Symbols as in Fig 1

was not performed for practical reasons Patient 3 a 50-year-old man with abdominal pains and diarrhea lacked a response despite no previous thyroid disorder All other thyroid tests were normal All his TSH levels during the TRH test were below detectable amounts Patient 4 a 55 year old housewife had paroxysmal tachycardia All additional blood tests were normal Her 24 hour radioiodine uptake was 32% She was followed for a year after which a new TRH test was performed and revealed normal values Patient 5 a 62 year-old housewife had a slight hypercalcemia All additional thyroid blood tests were normal Patient 6 a 64 year-old woman had palpitations Her additional blood tests were normal and the 24 hour radioiodine uptake decreased from 42 to 2% during the T_4 suppression test We regarded all the six patients as clinically euthyroid

Twenty of the 24 hyperthyroid patients did not respond to TRH and 4 responded slightly No hyperthyroid patient had a TSH response exceeding 3 0 μU/ml

Thus 77% of the normal subjects showed a normal TSH response to TRH i e an increment of more than 3 μU/ml A normal response to TRH ruled out hyperthyroidism A slight response to TRH was observed in 17% of the hyperthyroid patients

T_3 versus T_4 and TRH test

It is evident from Figs 1 and 2 and Table I that the T_3 determinations were the best discriminator between eu and hyperthyroidism as only one patient (2%) showed an overlap Seven (14%) of all the patients presented with an overlap with regard to the T_4 determinations The overlap for the TRH test was 10 patients (20%) if one accepts the criteria of a TSH increment of more than 3 0 μU/ml for normals and lack of response for hyperthyroid patients

Thus T_3 determination is the best discriminator between eu and hyperthyroidism followed by the determination of T_4 and the TRH test

The elderly age group The results from the group over 60 years of age did not differ substantially from those for the whole material as presented above

DISCUSSION

Thyroid disorders are a common clinical problem Their diagnosis is based upon a clinical examination and laboratory tests During the last two decades a battery of tests has been developed for this purpose comprising tests such as PBI BEI or T_4 iodine for the estimation of hormone levels by virtue of their iodine content or tests like resin T_3 uptake on the basis of hormone binding activities of serum proteins Other tests are based upon the estimation of the basal metabolic rate which assesses the energy exchange as measured by heat production or upon more direct measures of thyroid function conducted by studying the accumulation and release of radioactive iodine

The introduction of more sensitive tests has not often led to the abandonment of older methods Thus several tests have been used without an attempt to discriminate between their potential in the evaluation of thyroid function The rapid increase in laboratory costs required for the various tests has accentuated the need for a proper selection of tests especially with regard to the initial screening procedure The recent development of radioimmunoassay techniques for the estimation of thyroid hormones and TSH seems to provide a sufficiently sensitive and simple means of directly quantitating these substances in the serum in both normal and pathological states

In the diagnosis of primary hypothyroidism the clinical value of TSH determinations has now been well established (4) As regards the diagnosis of hyperthyroidism the clinical value of T_3 and T_4 as well as of the TRH test has been approved and found to be superior to the older tests (8) As yet however it is not entirely clear to what extent T_3 T_4 and the TRH test can replace each other, or whether all three tests are necessary in the initial screening of patients with possible thyroid hyperfunction

The ability of the TRH test to discriminate between euthyroid and hyperthyroid subjects has been studied by several investigators (6) It is generally agreed that hyperthyroid subjects fail to respond or show a weak blunted TSH response to TRH A similar response can also be found in euthyroid patients with the ophthalmic form of Graves disease in patients with previous Graves disease rendered euthyroid by treatment or in patients with autonomous thyroid nodules A failing or impaired response to TRH has also been described in normals (1 2 3 7) but a significant response is the most common finding

The results of the present investigation verify

that response to TRH may be missing even in normal individuals and an impaired response may occur both in normal and in hyperthyroid patients

The ability of the T_4 determination to discriminate between normal and hyperthyroid subjects is known to be less reliable than the determination of T_3 (8 10 12) and is also consistent with the present results Normal levels of T_4 were found in hyperthyroid patients and levels within the hyperthyroid range were found in normal subjects One reason for this overlap may be due to variations in hormone binding proteins This influence is at present under further investigation Another reason is that all hyperthyroid patients with normal T_4 levels had only slightly elevated T_3 levels and toxic goiters or solitary toxic adenoma. This combination has been described earlier (8)

The reliability of T_3 determination in discrimination between normal and hyperthyroid patients has been evaluated by many authors and remarkably little overlap has been found The results from the present investigation demonstrate a surprisingly good separation between normal and hyperthyroid patients Only one of the 50 subjects investigated demonstrated an overlap when the groups were separated by the 2 S D limit

In conclusion our results demonstrate that the determination of T_3 is superior to the determination of T_4 and the TRH test in the laboratory diagnosis of possible hyperthyroidism It seems to be reliable in the initial screening of patients with possible hyperthyroidism The TRH test is recommended as a complement if the level of T_3 is close to the upper normal limit A normal response to TRH does not indicate hyperthyroidism Further tests e g the T_3 or T_4 suppression test are recommended if response to TRH is absent or impaired

ACKNOWLEDGEMENTS

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Divergent Changes of Serum 3,5,3′-Triiodothyronine and 3,3′,5′-Triiodothyronine in Patients with Acute Myocardial Infarction

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ABSTRACT The serum levels of thyroxine (T_4) 3 5 3 triiodothyronine (T_3), 3,3 ,5 triiodothyronine (reverse T_3 rT_3), thyroxine-binding globulin and thyroid stimulating hormone have been monitored in 13 patients with acute myocardial infarction The major changes recorded were a transient decrease in T_3 and a transient increase in rT_3. They reached a nadir and a peak respectively, within three days A conceivable explanation for these alterations is that the monodeiodination of T_4 is diverted from the activating pathway (T_4 to T_3) to the inactivating pathway (T_4 to rT_3)

Several metabolic changes have been found in patients with acute myocardial infarction (AMI) They include increased blood and urine concentrations of catecholamines and corticosteroids (1 8 9 12 13 14 19) There is evidence that both catecholamines and corticosteroids can affect the production of thyroid hormones (6 7 9) and it is well known that these hormones influence myocardial activity Recently it has been demonstrated that during fasting (20) surgery (14) and in various non thyroidal illnesses (2 3 5 6 18) the serum concentration of the most active thyroid hormone 3 5,3 triiodothyronine (T_3) is reduced whereas that of its metabolically inactive counterpart 3 3 ,5 triiodothyronine (reverse T_3 rT_3) is enhanced This metabolic alteration appears to result from a shift in the proportions of T_3 and rT_3 generated by peripheral deiodination of thyroxine (T_4)

The present study concerns the possible alterations of thyroid hormone economy in patients with AMI This condition is of interest because it allows studies early during a disease with a sudden and well defined onset This study included analyses of the serum concentrations of T_4 T_3 rT_3 thyrotrophin (TSH) and thyroid hormone binding globulin (TBG) as well as determinations of in vitro uptake of T_3 The results of the present study suggest that in AMI patients the peripheral conversion of T_4 to T_3 is transiently reduced while that of T_4 to rT_3 is transiently enhanced

MATERIAL AND METHODS

The study was carried out on 13 patients (11 men and 2 women) aged 38–75 years (mean 60) admitted to the Central Hospital Vasterås due to AMI None had a personal or a family history or clinical signs of thyroid disease The diagnosis of AMI was confirmed by findings of typical chest pain ECG recordings and transient elevation of serum ASAT and ALAT The medication was confined to narcotic analgesics (10 cases) cardiac glycosides (2 cases) diazepam (5 cases) furosemide (5 cases) lidocaine (7 cases) and phenytoin (6 cases)

Blood samples were drawn by vein puncture at 7 p m on days 1 2 3 5 7 and 9 during the hospital stay The patients were selected so that the first blood sample always was taken less than 24 hours after onset of the chest pain

The serum levels of T_4 T_3 and TSH were determined with specific radioimmunoassay using commercially available reagents For the determination of T_4 Tetralab-RIA (Nuclear Medical Laboratories Dallas Texas USA) was used normal range 57–145 nmol/l T_3 was determined using T_3 RIA kit (Radiochemical Centre Amersham

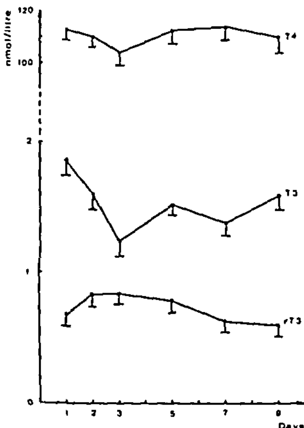


Fig 1 Serum T_4, T_3 and rT_3 concentrations (mean + S E M) Infarction day=day 1

UK) normal range 1 2-2 9 nmol/l Phadebas TSH kit (Pharmacia Uppsala Sweden) was used for the assay of TSH normal values <8 mU/l TBG was determined according to Nielsen et al (17) normal range 70-135% of a normal human serum pool T_3-resin uptake test was performed using *Sephadex* as absorbent according to Hansen (11) normal range 75-120% of the resin uptake from a sample of pooled normal human serum The serum levels of rT_3 were determined by radioimmunoassay without preceding extraction according to Nicod et al (16) normal range 0 38-1 00 nmol/l

The results from each day were compared with those of day 1 by Student s *t* tests Values are given with S E M within parentheses

RESULTS

Serum T_3, rT_3 and T_4

Within one day the serum T_3 levels were reduced from an initial mean of 1 86 (±0 12) nmol/l and reached a minimum of 1 24 (±0 12) nmol/l on the third day after admission (Fig 1) The difference was highly significant ($p<0$ 001) The serum levels of rT_3 displayed an opposite pattern they increased within one day from an initial mean of 0 68 (±0 09) nmol/l and reached a maximum of 0 83 (±0 09) nmol/l on day 2 which persisted over day 3 (0 83±0 07 nmol/l) (Fig 1) The mean values on days 2 and 3 were significantly higher ($p<0$ 05) than on day 1 Both the decrease in T_3 and the increase in rT_3 were transient normal levels were recorded within 9 days (Fig 1)

The mean numerical value of serum T_4 (Fig 1) showed a slight decrease but the difference between the initial and the lowest (day 3) mean values was not significant

Serum TBG and in vitro uptake

The mean serum concentration of TBG was significantly reduced on day 3 ($p<0$ 05 vs day 1) and then rose towards values not significantly different from the initial level (Fig 2) The T_3 uptake test values displayed no significant alterations (Fig 3)

Serum TSH

The mean serum concentration of TSH was significantly higher ($p<0$ 05) on day 2 than on day 1 but then returned towards initial values (Fig 4)

DISCUSSION

The major finding in this study was the early decrease and increase respectively in serum T_3 and rT_3 Among the drugs administered phenytoin is known to promote alterations in the serum levels of thyroid hormones (10) As phenytoin was given to only 6 of the 13 patients and as no other drug was given to all patients it is not likely that the recorded alterations were evoked by the medication Hence *it is assumed that the changes were consequent to* the illness Indeed reduced T_3 and enhanced rT_3

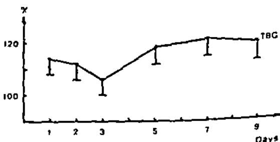


Fig 2 Serum TBG concentrations (mean +S E M) Infarction day=day 1

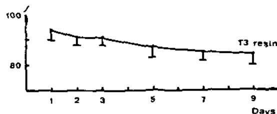


Fig 3 Saturation of T_3-binding plasma proteins measured with the in vitro T_3 uptake test on serum (mean +S E M) Infarction day=day 1

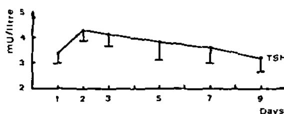


Fig 4 Serum TSH concentrations (mean+S E M) Infarction day=day 1

serum levels have been observed during illnesses of other kinds (2 3 5 6 18) as well as during fasting (20) and following surgical operations (4) In the present study the T_3 reduction appeared to be large enough to promote a transiently and slightly increased secretion of TSH This agrees with observations on patients with different illnesses (2 18)

One probable common denominator in these different conditions is the reduction or absence of food intake and another one is an increased secretion of glucocorticoids and an increased tissue catabolism In this context it is of interest that an increased exposure to glucocorticoids in healthy volunteers as well as in T_4 substituted patients promotes decreased T_3 and increased rT_3 serum levels (7 9)

Recent investigations have shown that in addition to T_4 both T_3 and rT_3 are secreted by the normal human thyroid gland (21) However the major parts of both T_3 and rT_3 are generated outside the gland by peripheral deiodination of T_4 (21) Moreover changes in the thyroidal exposure to TSH seem to be followed by similar, not divergent alterations in the secretion of T_4 T_3 and rT_3 (21) Therefore the present finding of reduced T_3 and enhanced rT_3 levels can hardly be secondary to a change in the thyroidal secretion of iodothyronines

The most important transport protein for iodothyronines is TBG The TBG levels were slightly reduced during the first days probably as a result of expansion of plasma volume due to the recumbent body position Even though the reduction of TBG could help to explain the decrease in serum T_3 it is not the most likely cause as rT_3 and T_4 would then also be reduced As this was not the case it is not probable that changes in the distribution of the iodothyronines could explain the findings Accordingly the most reasonable explanation of the opposite changes in the T_3 and rT_3 concentrations is a shift in the proportions of T_4 which are deiodinated to T_3 and rT_3 respectively Such a shift is also the most probable cause of the T_3/rT_3 changes recorded in subjects exposed to fasting malnutrition illnesses of various kinds and to increased levels of glucocorticoids (see above)

Irrespective of the mechanism whereby the serum T_3 and rT_3 levels are changed in AMI patients the (patho-) physiological and clinical significance of these changes remains to be determined A major threat to the patients during the first days of an AMI emerges from ventricular ectopic rhythms It is not unlikely that variations in thyroid hormone concentrations may influence the appearance of such rhythms but it is not known whether the changes recorded in the present study represent teleologically adequate reactions or not

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Hypomagnesaemia and Muscle Electrolytes and Metabolites

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ABSTRACT Ten patients aged 39–61 years, with hypomagnesaemia due to chronic alcoholism (7 cases) or malabsorption (3 cases), have been investigated by assessing the maximum isometric voluntary contraction force (MVC) of the quadriceps femoris muscle (7 cases), laboratory screening (9 cases) and estimating the electrolyte and metabolite content of biopsy specimens from the quadriceps femoris muscle The MVC ranged from 0 5 to 34 kp and was significantly lower than in 12 apparently healthy normomagnesaemic controls ($p<0\ 001$) The results of the laboratory screening apart from a significant lowering of the serum magnesium concentration ($p<0\ 01$), were mainly within the range of normal values, apart from signs of liver damage, such as an elevated activity of S OCT (3 cases) alkaline phosphatase (3 cases) S ALAT (1 case) and an elevation of bilirubin and blood ammonia (2 cases) Low serum iron binding capacity occurred in 4 cases a finding reported in protein–calorie malnutrition Muscle magnesium content was significantly lower than in healthy controls ($p<0\ 001$) Muscle sodium and chloride contents were significantly increased ($p<0\ 05$) Total H_2O content and the extracellular H_2O content were both significantly increased ($p<0\ 05$) Pyruvate and lactate values were within the normal range The apparent equilibrium constant for creatine kinase differed significantly ($p<0\ 01$) ATP values were within the normal range, but there were slight decreases for ADP ($p<0\ 05$) and creatine phosphate ($p<0\ 01$), which is of interest in view of the lowering of the MVC and the diminished capacity for sustained muscular effort in hypomagnesaemic patients reported earlier

Segmental muscle necrosis has been reported in chronic alcoholism in the acute muscular syndrome which follows an intensive alcohol debauche (11 15 20 24 38 40 44) A chronic form of muscular myopathy in the alcoholic presenting chiefly as muscular weakness and atrophy particularly in the proximal muscle group has been described (11 14 20 24 38 41) Subclinical muscle damage in the alcoholic was evidenced by Nygren (35) and lowering of the isometric (static) muscle strength in the proximal muscles particularly in the quadriceps femoris muscle in chronic alcoholics by Carlsson (7) and Carlsson et al (8) A lower isometric muscle strength in patients with hypomagnesaemia both alcoholics and non alcoholics compared with normomagnesaemic alcoholics and normal controls was reported by Stendig Lindberg (42) The whole spectrum of alcoholic myopathies has been reviewed by several workers (19 23 35 37) A number of authors have reported hypomagnesaemia in the alcoholic beginning with Flink et al (16)

The description of the morphological findings in homo in the myopathies of chronic alcoholic in whom magnesium deficiency is documented suggested a resemblance to the morphological findings in the muscle of the experimental animal fed a magnesium-deficient diet (21 28 31) The question arose therefore whether magnesium deficiency per se could be a causal factor in the myopathies seen in alcohol disease as well as in those seen in patients with other diagnoses and hypomagnesaemia

In order to answer this question we investigated ten patients seven with the diagnosis of alcoholismus chronicus and three with the diagnosis of malabsorption and with signs of muscular weakness and hypomagnesaemia (serum magnesium concentration of ≤0 72 mmol/l) Three patients in the alcoholic group and one in the malabsorption

Table 1 *Sex age serum magnesium concentration (first sampling) maximum voluntary contraction force (MVC) and the diagnoses*

Pat. no	Sex	Age (y)	Quadriceps femoris muscle isometric MVC (kp) Right	Left	Serum magnesium concentration (mmol/l)	Diagnoses
1	♀	44	–	–	0 68	Alcoholismus chronicus cirrhosis hepatis haematemesis+ melaena (oesophageal varices) resectio lobus caudatus hepatis+ portocaval shunt operation
2	♀	44	–	–	0 65	Alcoholismus chronicus cirrhosis hepatis haematemesis melaena (oesophageal varices) epistaxis splenorenal shunt operation+ spleen extirpation
3	♂	54	–	34	0.57	Alcoholismus chronicus+fractura calcanei dx+fractura tibiae et fibulae sin+epilepsia+hypertonia essentialis benigna+persona pathologica paranoides
4	♀	50	0.5	1	0 68	Status post operation for colon diverticulitis with perforated abscess +milk intolerance+diarrhoea +malabsorption+ psoriasis with joint affections+osteopoenia (plain X-ray diagnosis)+muscular weakness & atrophy+polyneuritis+urinary tract infection
5	♀	59	14	13	0 63	Fistula jejuno-ilealis+malabsorption
6	♂	50	26	26	0.57	Alcoholismus chronicus+ pleuritis dx pulmonary tuberculosis+proteinuria
7	♂	61	26	23	0 42	Alcoholismus chronicus+hypertonia essentialis+incompensatio cordis
8	♂	39	14	19	0 70	Pancreatitis chronica recidivans+status post resectio ventriculi et status post resectio pancreatis+functio laesa et malabsorptio+status post abusus alcoholica+epilepsia (tarda?) NUD
9	♂	41	–	–	0 65	Alcoholismus chronicus+epilepsia
10	♂	64	27	35	0 71	Alcoholismus chronicus+epilepsia+ atelectasis (tuberculosis?)

* Reading omitted because of residual pain

group received magnesium treatment per os prior to the examination in order to further isolate the possible role of magnesium

The object of this investigation was to find out whether patients with hypomagnesaemia display any common feature as regards electrolyte and metabolite muscle content

MATERIAL AND METHODS

Patient material

The material consisted of 4 female aged 44–59 years (mean 49 3 S E M 3.5) and 6 male aged 39–61 years (mean 51.5 S E.M 4.2) patients at Karolinska Hospital All complained of muscular weakness confirmed at physical examination All had serum magnesium concentrations of ≤0 71 mmol/l at the first examination The diagnoses are listed in Table 1

The patients were divided into four groups. I) Hypomagnesaemic alcoholics not treated with magnesium (nos 1 2 3 10) II) Hypomagnesaemic alcoholics on magnesium replacement therapy per os (nos 6 7 9) III) Hypomagnesaemic malabsorption patients not treated with magnesium (nos 4 8) IV) Hypomagnesaemic malabsorption patient on magnesium replacement therapy per os (no 5)

Magnesium treatment

Four patients (nos 5 6 7 9) received magnesium replacement treatment per os Patient 5 received magnesium

chloride capsules containing 1 88 mmol magnesium each and the others received magnesium oxide mixture (Nordic Pharmacopoeia) containing 1 20 mmol magnesium/ml

Physical examination and measurements of MVC

All patients underwent a physical examination with special emphasis on their muscular status Measurement of the isometric maximal voluntary contraction force (MVC) of the quadriceps femoris muscle was carried out in seven cases by the modified method of Tornvall (43) (Table I)

These measurements were compared with those performed in 12 apparently healthy normomagnesaemic controls aged 21–56 years (mean 31 1 S E M 3 4) The mean MVC of the controls was right side 52 kp S E M 2 8 (range 41–65) left side 54 kp S E M 2 6 (range 42–68)

Laboratory examination and biopsy technique

Serum magnesium concentration was estimated by atomic absorption spectrophotometry at the Clinical Chemistry Laboratory of Karolinska Hospital using a Perkin Elmer spectrometer No 403 (variation coefficient 1 18% at the 95% level) Simultaneous serum laboratory screening was carried out at the same laboratory in four cases and at the Central Laboratory of St Erik s Hospital in six cases In these six cases the serum creatine kinase (S CK) was estimated at the Laboratory of Clinical Chemistry Sabbatsberg Hospital by the method of Oliver (36) see Nygren (34) and the ornithine carbamoyl transferase (S OCT) by the method of Reichard (39) Laboratory screening was performed on the same day as the biopsy except in case 4 (6 days earlier) (Table II)

In 6 cases muscle metabolites and electrolytes were examined at the Research Laboratory St Erik s Hospital Muscle samples were taken after 2–8 days following the MVC measurement in five cases after 30 days in one case (no 9) The subjects had been fasting overnight and their informed consent had been obtained Samples were collected at 8–10 a m after a rest for at least 20 min from the quadriceps femoris muscle with a biopsy needle according to the method of Bergström (2)

One sample for metabolite determination was frozen immediately after withdrawal from the muscle by plunging the biopsy needle into liquid freon maintained at the melting point (−150°C) Extraction and determination of metabolites were carried out according to the methods of Harris et al (18) Pyruvate ADP and AMP were analysed by a fluorometric method (29)

A separate specimen for water and electrolyte determination was taken from the same muscle 4–5 cm more proximally One of the specimens which weighed 20–80 mg was divided into 2–4 pieces of 10–20 mg Visible fat and connective tissue were rapidly removed by dissection and the specimens were weighed on an electromagnetic balance The specimens were dried at 90°C and weighed Neutral fat was extracted with petroleum ether the pieces were weighed again and the water and fat contents were calculated The details of the biopsy technique weighing procedure and fat extraction have been described earlier (2) Sodium potassium and magnesium contents were determined by atomic absorption spectrophotometry and chloride was measured by electrometric titration (4) Tissue water and electrolyte contents were referred to 100 g fat free solids

The determination of extra and intracellular water was based on the chloride method Chloride is freely diffusible across the skeletal muscle fibre membrane and is distributed according to Nernst s equation (10) Taking the resting membrane potential of muscle in normal man to be 87 2 mV (5) the Cl_e/Cl_i ratio calculated from Nernst s equation will be 26/1 if the total water and chloride content of the muscle tissue and the extracellular concentration of chloride (obtained by correcting the plasma chloride concentration for a Donnan factor and a factor for plasma water (2)) are known extra and intracellular water volumes and intracellular electrolyte concentrations can be calculated (3 17)

RESULTS

The maximum isometric voluntary contraction force of the quadriceps femoris muscle

The mean MVC on the right side was 17 9 kp (S E M 4 3 $n=6$) on the left side 21 6 kp (S E M 4 5 $n=7$) in the patients tested (range 0 5–35 kp) (Table I) The MVC was significantly decreased compared with the normal controls ($p<0\ 001$ Student s t test)

Laboratory screening The results given in Table II showed lowering of the serum magnesium concentration ($p<0\ 01$) but were otherwise mainly within what is considered the normal range except for the following deviant values in the six patients who underwent a biopsy *Case 5* increased alkaline phosphatase and lowered serum iron concentration *Case 6* increased S-OCT and lowered total iron binding capacity (TIBC) *Case 7* lowered serum calcium concentration and TIBC *Case 8* increased S OCT lowered TIBC and lower borderline value for total protein *Case 9* rise of S ALAT and lowered TIBC *Case 10* increased S OCT For deviant values in cases 1–4 see Table II

Muscle electrolytes The results are shown in Table III The mean Mg^{++} content per 100 g fat free solids was significantly lowered compared with the controls ($p<0\ 001$) The lowest values were found in patients 7 and 9 in spite of magnesium treatment per os of 2 and 11 days duration respectively (which in the latter case normalized the serum magnesium concentration) Mean Na^{+} and Cl content and total and extracellular water content per 100 g fat free solids were significantly raised ($p<0\ 05$) The magnesium/potassium quotient (2) was significantly lowered ($p<0\ 001$)

Table II *Laboratory screening of the patients investigated on the day of biopsy*

	Pat no									
	1	2	3	4	5	6	7	8	9	10
S magnesium (mmol/l)	0 68*	0 65*	0 57*	0 67*	0 70	0 50*	0 50*	0 70*	0 85	0 70*
S-calcium (mmol/l)	2 5	–	–	2 37	2 28	2 48	2 08*	2 28	2 38	2 45
S phosphate (mmol/l)	–	–	–	5 3*	3 6	4 7	3 3	4 5	–	4 0
S potassium (mmol/l)	*4 1*	–	–	*4 5*	*4 0*	*4 2*	*4 2*	*3 9*	*3 8*	*4 3*
S sodium (mmol/l)	134*	–	–	140	142	145	145	146	141	140
S-chloride (mmol/l)	102	–	–	–	109	105	103	107	107	104
B standard bicarbonate (mmol/l)	22	–	–	–	–	–	–	–	–	–
B-ammonia ion (μmol/l)	116*	144*	–	–	–	–	–	–	–	–
S-creatinine (μmol/l)	44[b]	–	–	97	62	80	106	62	97	97
S protein (g/l)	88*	67	–	–	61	66	62	60[b]	72	69
S albumin (g/l)	45	38	–	–	34	36	32	33	39	42
S iron (μmol/l)	–	–	–	13 4	9 5*	26 7	27 4	16 8	23 1	16 6
S TIBC (μmol/l)	–	–	–	53 1	45 7	44 3*	41 8	43 4*	36 9	53 9
B-sugar (mmol/l)	–	7 2*	–	–	4 7	–	3 7	4 1	–	–
S bilirubin (μmol/l)	49 6*	30 8*	–	–	5 1	5 1	–	–	5 1	13 7
S ASAT (U/l)	19	22	–	7	13	22	19	26	29	19
S ALAT (U/l)	12	10	–	4	15	16	15	33	41*	16
S LDH (U/l)	–	–	–	207	130	177	209	132	165	176
S ALP (U/l)	10 [illegible]	7	–	28 [illegible]	3 1	2 5	1 9	2 6	2 2	1 5
S-OCT (U/l 24 C)	–	–	–	–	<6	8*	–	7*	1	6*
S CK (U/l 35 C)	–	–	–	–	18	42	12	12	28	14
Zinc sulphate reaction (U)	–	–	–	–	2	6	6	4	7	4
Triglycerides (mmol/l)	–	–	–	3 2*	–	–	–	–	–	–
S-cholesterol (mmol/l)	–	–	–	7 5	5 0	5 2	4 9	5 6	5 0	7 3

* Deviant values [b] Borderline values Buch units (normal values 2–8)

Metabolites in muscle The results are shown in Table IV Mean creatine phosphate was significantly lowered in the patients compared with the controls ($p<0\ 01$) as was the mean ADP content ($p<0\ 05$) The apparent equilibrium constant for CK reaction was significantly increased ($p<0\ 01$)

DISCUSSION

The MVC was significantly lowered confirming earlier findings in hypomagnesaemic subjects (47) Apart from the significant lowering of the serum magnesium concentration the laboratory findings

Table III *Serum magnesium muscle water and electrolytes in 6 patients and 10 normal subjects*

Pat no	Serum Mg (mmol/l)	mmol/100 g fat free solids (FFS)				ml/100 g FFS			g/100 g FFS
		Mg	K	Na	Cl	H_2O_{tot}	H_2O	H_2O_i	Fat content
5	*0 70*	*4 4*	*48 3*	*9 2*	*5 9*	344	37	307	5 2
6	0 50	4 2	48 7	9 0	7 4	352	52	300	7 6
7	0 50	3 8	43 4	18 1	14 4	372	117	255	22 0
8	0 70	3 5	36 1	29 0	22 0	394	177	217	17 3
9	0 85	3 9	46 7	13 0	8 2	345	57	287	27 2
10	0 70	4 4	47 7	10 0	6 9	344	48	296	13 3
Mean	0 658	4 00	45 15	14 72	10 80	358 5	81 4	277 0	12 93
S D	0 135	0 30	4 82	7 79	6 26	20 4	54 6	34 6	9 87
S E M	0 055	0 15	1 97	3 18	2 55	8 3	22 3	14 1	4 02
Range	0 50–0 85	3 9–4 4	43 4–48 7	9 0–29 0	5 9–22 0	344–394	48 1–177	217–307	5 2–27 2
Normals (n=10)									
Mean	0 84	4 48	46 7	9 3	6 0	336	39 9	297 0	6 4
S D	0 04	0 08	0 87	1 52	1 19	11 78	10 11	8 6	3 10
S E M		0 03	0 27	0 48	0 38	3 72	3 20	2 7	0 98
p	<0 01	<0 001	>0 05	<0 05	<0 05	<0 05	<0 05	>0 05	>0 05

p = Significance of differences between the means of the patients and the controls (Student s *t* test)

	SD	SEM	Normal range
)	0 105	0 033	0 73–0 90
)	0 139	0 049	2 20–2 60
	0 74	0.30	2 2–5 0
	0 23	0 08	3 4–5 1
,	3 9	1 4	136–148
)	2.5	0 9	95–110
			19–25
			<100
	22.3	7 9	44–141
	9 0	3 2	60–82
	4.5	5.2	>28
7	6 82	2 58	11 0–36 0
3	5 87	2 21	45–75
	1 59	0 80	3 3–5 7
3	18.33	7 48	3 4–17 1
	6 6	2 2	<40
	11 6	3 9	<35
9	31 7	12 0	<225
	0.56	0 23	<3 0
	2 7	1.2	<6
	11.5	4 7	0–100
	1 8	0 7	<12
			<1 70
	1 13	0 43	3 9–7 7

were within the normal range apart from the tendency to a lower TIBC found in 4 cases

Serum calcium concentration was lowered in one case only (no 7) in whom the other findings were within the normal range (except for low TIBC)

Elevated activity of S OCT and of alkaline

…ived values (mmol/l intracellular water)

	Na_i	K_i^+	Mg^+/K^+
1	12 8	157 2	0 090
8	4 5	164 6	0 085
5	4 6	162 9	0 086
3	20 1	165 7	0 096
4	12.5	157 0	0 084
8	11 1	161 1	0 082
32	10 93	161 42	0 0888
69	5 85	3 69	0 0046
78	2 39	1.51	0 0019
4–15 3	4 5–12 8	157 0–164 6	0 084–0 096
96	12 6	157 3	0 095
51	2 04	4 50	0 003
16	0 65	1 42	0 001
05	>0 05	>0 05	<0 001

phosphatase in 3 cases each of S-ALAT (in one case) as well as the raised serum bilirubin and blood ammonia in two patients speak in favour of the presence of impaired liver function

Only a few studies of muscle magnesium content in man are to be found in the literature Some authors report a low muscle magnesium content in hypomagnesaemia others have found concentrations that are considered to lie within the normal range (1 6 12 25 26 27 30 32)

Our results show that patients with hypomagnesaemia appear to have a low muscle magnesium content both when estimated with reference to fat free solids and to muscle potassium content (2) A decrease in magnesium in relation to fat free solids might be explained by an increase in connective tissue content since connective tissue is known to have a low content of intracellular electrolytes i e potassium and magnesium However an increase in connective tissue content is ruled out as the only explanation by the fact that magnesium was also low in relation to the potassium content

The relative decrease in muscle magnesium amounted to only 11% This is not surprising in view of the fact that only a small fraction of total magnesium in muscle tissue is present in free ionized form the rest being bound as a complex with ATP and various metabolites (33) Thus even a substantial decrease in intracellular free magnesium content might appear only as a very moderate decrease in total magnesium content provided the bound magnesium fraction remains unchanged It is probable that the free magnesium content is an important factor for the neuromuscular excitability of the muscle fibres (9) and possibly also for the electrolyte transport

The increase in total water content per 100 g fat free solids was a consequence of an increase in the extracellular water content the intracellular water content remaining normal or low

Sodium and chloride which are predominantly extracellular electrolytes also accumulated in muscle tissue The intracellular sodium concentration was low in two cases and high in one Retention of sodium with an expansion of extracellular fluid is a common response to trauma and disease and this accumulation is reflected in muscle tissue as well Since these patients all had a history of chronic disease these changes cannot be attributed to magnesium deficiency alone

We found a lowering of the mean

Table IV *Energy rich phosphagens and metabolites (mmol/kg dry muscle) in 6 patients and normal controls*
PC=creatine phosphate Cr=creatine

Pat no	ATP	ADP	AMP	PC	Cr	Pyruvate	Lactate
5	23 8	3 1	0 2	75 8	69 1	0 2	1 9
6	25 3	3 5	0 2	68 6	53 0	0 2	1 9
7	19 0	2 3	0 1	46 0	41 2	0 5	6 6
8	23 6	2 9	0 1	69 6	55 7	0 4	2 3
9	20 1	2 4	0 1	62 5	50 9	0 2	2 7
10	26 9	3 1	0 2	68 9	46 4	0 3	3.5
Mean	23 10	2 89	0 15	65 21	52 71	0 30	3 15
S D	3 05	0 46	0 02	10 33	9.52	0 10	1 77
S E M	1 24	0 19	0 01	4.22	3 89	0 04	0 72
Range	19 0–26 9	2.3–3 5	0 1–0 2	46 0–75 8	41 2–69 1	0 2–0.5	1 9–6 6
Normals							
n	81	66	50	81	81	66	81
Mean	24 0	3 2	0 1	75 5	49 0	0 4	5 1
S D	2 64	0 45	0 05	7 63	7 62	0 21	2 47
S E M	0 29	0 05	0 01	0 84	0 84	0 02	0 27
Range ($x\pm2$ S D)	18 7–29 3	2 4–4 2	0–0 2	60 2–90 8	33.8–64 2	0–0 9	2 6–7 6
p	>0 05	<0 05*	*	<0 01*	>0 05	>0 05	>0 05

p=Significance of differences between the means of the patients and the controls (Student s *t* test)
* The possible difference could not be ascertained statistically because the mean of the controls could not be expressed with an accuracy of more than 1 decimal point (owing to the limited precision of the photometric method used (18))

ith the most marked decrease in case 7 in whom total creatine was also decreased The latter patient also showed the lowest magnesium and the highest chloride content in muscle This may be a sign of a relative decrease in muscle fibre content in the biopsy specimen which could be explained by protein-calorie malnutrition resulting in net catabolism of muscle protein Such malnutrition is also known to give decreased serum iron binding capacity (13) this was observed in our patients 6 7 8 and 9 A decrease in white muscle fibre protein in alcoholics was observed by Kiessling et al (22)

Except for the slight lowering of creatine phosphate and a small decrease in ADP content all metabolites measured showed normal values These minor changes in metabolite content do not suffice to explain the muscle weakness found in patients with hypomagnesaemia

However it should be pointed out that these findings represent steady-state values at rest and cannot portray any metabolic alterations which might follow exertion

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PC+Cr	ATP+ ADP+ AMP	ATP×AMP / ADP²	ATP×Cr / ADP×PC
144 9	27 1	0 40	6 99
121 6	29 0	0 35	5 56
87 6	21 5	0 51	7 35
125 3	26 6	0 41	6 49
113 4	22 5	0 40	6 76
115 3	30 1	0 42	5 85
117 93	26 13	0 415	6 500
18 81	3 46	0 052	0 684
7 68	1 41	0 021	0 279
87 1–144 9	21.5–30 1	0 35–0 42	5 56–7 35
81	50		54
124 4	27 4		4 98
11 21	2.52		1 16
1 24	0 35		0 16
>0 05	>0 05		<0 01**

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Lactose Malabsorption and Bone Mineral Content

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ABSTRACT Bone mineral has been measured by the Am 241 gamma ray attenuation method in 12 men and 22 women with lactose malabsorption and in 17 men and 17 women with normal lactose absorption and compared with previously established normal values Although lactose malabsorption led to reduced milk consumption and calcium intake it did not cause osteoporosis

Lactose in the diet improves calcium absorption (5) Some people—17% in Finland (11 13)—have an isolated lactase deficiency and a number of them do not tolerate milk the main source of calcium Some cases of osteoporosis might be explained on the grounds that patients with low intestinal lactase activity drink much less milk than healthy individuals (3 6) and hence ingest less calcium throughout their lives

In this study we measured the bone mineral density by Am 241 gamma ray attenuation in patients with lactose malabsorption (LM) to establish whether or not they were osteoporotic

PATIENTS AND METHODS

The patient group (12 men and 22 women) had LM and the control group (17 men and 17 women) had normal lactose absorption They were all admitted to the Outpatient Clinic of University Central Hospital Kuopio with abdominal complaints Patients with general malabsorption and those who had diseases such as rheumatoid arthritis that are known to affect bone mineral metabolism were excluded (15) The mean age of the men in the LM group was 42 years (range 24–68) and in the control group 41 (range 21–72) the corresponding figures for the women were 40 (range 19–64) and 43 (range 20–71) The age distributions of the LM and control groups were nearly identical for both sexes (χ^2 test patients grouped at ten year intervals $\chi^2=3\ 21$ $p<0\ 60$ for males and $\chi^2=5\ 67$ $p<0\ 40$ for females)

A lactose tolerance test was performed after overnight fasting (about 10 hours) The subjects were given 1 0 g/kg lactose by mouth as a 20% solution Capillary blood samples were taken before and after 20 40 and 60 min for determination of blood glucose concentrations (10) The criterion for LM was a maximal rise of less than 1 3 mmol/l in blood glucose concentration

The serum calcium (normal values 2 30–2 75 mmol/l 8) serum phosphorus (normal values 0 81–1 45 mmol/l 7) serum alkaline phosphatase (normal values 107±38 4 for women and 121±57 9 U/l 37 C for men 2) and serum total protein (normal values 67–77 g/l) were determined

Bone mineral measurements were made by the Am 241 gamma ray attenuation method (12) in the nondominant forearm in the distal radium and at the point between the middle and distal thirds of the radius and ulna The results were analyzed statistically in comparison with normal values (1) The normal values were taken at a point corresponding to the patient s age from the third degree regression curve for the nondominant forearm

RESULTS

The results of the mineral measurements are given in Table I The mineral densities of the distal radius do not differ significantly from normal in either the patients with LM or those with normal lactose absorption At the cortical radius and ulna there are some statistically significant differences both the LM and the control patients apparently having more mineral than the normal population The mineral contents at different measuring sites show no statistically significant differences between the LM and the control groups

The mean serum calcium phosphorus alkaline phosphatase and total protein concentrations of the LM and the control groups do not differ significantly (Table II)

Of the 34 LM patients 70% did not drink milk at all only 2 consumed more than one glass of milk per day The average milk consumption of the controls was 2 glasses per day

Table I *Bone mineral linear density (λ) mineral density (ρ) and linear density/bone width (λ/d) in patients and controls and corresponding normal values*

The lower indices of the mineral content symbols denote 1=distal radius 2=midshaft radius 3=midshaft ulna

	Mineral content	Patients (Mean ±1 S D)	Normals (Mean ±1 S D)	*t*	*p*
Males					
LM	λ_1 (g/cm)	1 71 ±0 23	1 68 ±0 14	0 56	NS
(*n*=12)	ρ_1 (g/cm³)	0 325±0 029	0 314±0 015	1 47	NS
	λ_2 (g/cm)	1 55 ±0 24	1 47 ±0 07	1 30	NS
	λ_2/d (g/cm²)	1 01 ±0 07	0 98 ±0 04	1 55	NS
	λ_3 (g/cm)	1 30 ±0 13	1 26 ±0 06	0 98	NS
	λ_3/d (g/cm²)	1 08 ±0 08	1 04 ±0 04	1 67	NS
Controls	λ_1 (g/cm)	1 71 ±0 23	1 60 ±0 09	1 15	NS
(*n*=17)	ρ_1 (g/cm³)	0 317±0 034	0 315±0 013	0 25	NS
	λ_2 (g/cm)	1 49 ±0 12	1 49 ±0 09	0 02	NS
	λ_2/d (g/cm²)	1 03 ±0 07	0 98 ±0 06	2 47	<0 05
	λ_3 (g/cm)	1 28 ±0 25	1 28 ±0 08	0 05	NS
	λ_3/d (g/cm²)	1 08 ±0 12	1 05 ±0 05	1 11	NS
Females					
LM	λ_1 (g/cm)	1 10 ±0 17	1 09 ±0 07	0 25	NS
(*n*=22)	ρ_1 (g/cm³)	0 288±0 039	0 286±0 013	0 34	NS
	λ_2 (g/cm)	1 03 ±0 12	0 99 ±0 06	1 13	NS
	λ_2/d (g/cm²)	0 86 ±0 06	0 82 ±0 05	2 49	<0 025
	λ_3 (g/cm)	0 83 ±0 10	0 81 ±0 06	0 59	NS
	λ_3/d (g/cm²)	0 92 ±0 08	0 88 ±0 05	2 24	<0 05
Controls	λ_1 (g/cm)	1 07 ±0 18	1 08 ±0 08	−0 15	NS
(*n*=17)	ρ_1 (g/cm³)	0 263±0 034	0 281±0 018	−2 06	NS
	λ_2 (g/cm)	1 01 ±0 16	0 99 ±0 07	0 73	NS
	λ_2/d (g/cm²)	0 85 ±0 09	0 82 ±0 06	1 34	NS
	λ_3 (g/cm)	0 80 ±0 12	0 81 ±0 05	−0 29	NS
	λ_3/d (g/cm²)	0 87 ±0 11	0 87 ±0 06	0 02	NS

The *t* test and paired comparison were used for the statistical analysis (NS=*p*>0 05)

DISCUSSION

The LM group and control group were comparable in age The former drank less milk than the latter There were no statistically significant differences in the means of their serum calcium phophorus alkaline phosphatase and total protein concentrations The mineral measurements did not show the LM patients to be osteoporotic

The relationship between osteoporosis and calcium deficiency presents several contradictions

Table II *Laboratory findings*

	LM		Controls			
	n	Mean ±1 S D	*n*	Mean ±1 S D	*t*	*p*
Males						
Calcium	12	2 40±0 16	16	2 51±0 16	−1 72	NS
Phosphorus	11	1 04±0 _0	14	1 00±0 18	0.57	NS
Alkaline phosphatase	9	164 ±28	16	146 ±49	1 16	NS
Total protein	7	73 0±4 2	7	72 0±1 6	0 22	NS
Females						
Calcium	22	2 49±0 16	15	2 44±0 14	1 10	NS
Phosphorus	21	1 03±0 13	14	1 08±0 16	−0 95	NS
Alkaline phosphatase	21	147 ±47	17	130 ±33	1 35	NS
Total protein	13	73 1±4 4	12	71 3±2 6	1 40	NS

The statistical analysis was made with the *t* test for two means NS=*p*>0 05

Dietary deficiency of calcium causes osteoporosis in growing animals (9) Although this has not been shown to occur in man calcium is frequently considered in the treatment of osteoporosis

Milk is man s main source of calcium People with lactase deficiency get abdominal complaints after drinking milk and tend to avoid it so they ingest less calcium than individuals with normal lactose absorption and may also absorb less of what they do ingest because lactose increases calcium absorption (5) So if calcium intake and absorption play any part in causing osteoporosis one might expect to find the disorder in patients with LM

On the other hand the prevalence of LM is lower in white people than in negro and yellow populations while milk consumption is high in white populations and low in the others (14) But the incidence of osteoporosis is high in populations with high milk consumption (4) These contradictions could be explained by some unknown cause of osteoporosis by calcium sources other than milk or by racial differences

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S-Tryptophan Concentrations after Intestinal Bypass in extreme Obesity

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ABSTRACT **Treatment of extreme obesity with je junoileostomy was followed by a decreased level of S tryptophan permanently low concentrations were recorded postoperatively in 29 out of 52 patients Patients in the low tryptophan group had a higher rate of weight loss and a higher incidence of electrolyte disturbances and s gns of l ver injury Symptoms of depression and anxiety were sl ghtly more common in patients with low S tryptophan The influence of a decreased S albumin and a deranged amino acid pattern on the non protein bound fraction of S-tryptophan needs further investigation Serum levels of tryptophan rose significantly after two weeks oral supplementation with 1 2 g L-tryptophan daily this dosage was insufficient to normalize a low S tryptophan level in patients who have undergone jejunoileostomy**

Intest nal bypass operat ons have been performed for more than 20 years in order to induce we ght loss in obese pat ents and to prevent compl cations of hyperlip dem c disease The metabol c consequences of the postoperat ve malabsorptive state are partly known and changed serum patterns of both l pids (16) and am no ac ds (3) have been reported

Our interest was focused on the essent al amino acid tryptophan because its absorpt on is interfered w th in various other malabsorptive disorders (11 17) and because of its possible l nk to mental depression (5 6 ?0) which is a common psych atric complication follow ng intestinal shunt operat on (1?) The aim of the present nvest gat on was to exam ne serum levels of tryptophan in pat ents operated on with jejuno leostom es Since decreased serum levels were found in a cons derable number of patients the study was extended to comprise an analysis of the postoperative courses in patients with tryptophan deficiency The frequency of psy ch atric symptoms and the r poss ble relation to a low serum level of tryptophan were analysed and the serum levels during supplementat on with a moderate da ly dosage of tryptophan were ex am ned

METHODS

Su g cal proced es Two types of jejuno leostom es were performed In 8 pat ents the anastomos s was made end to-s de (Fig 1 ?) accord ng to Payne and De W nd (?3) and n 44 pat ents t was made end to-end w th ileocecostomy (F g 1 3) accord ng to Buchwald et al (4) The small intest nal length was measured ntraoperatively accord ing to Backman and Hallberg (?)

Calculat on of body we ght k net cs To fac l tate com parison between nd v duals all body we ghts were ex pressed in relat ve terms accord ng to Broca s index

$$\frac{\text{Body we ght (kg)}}{\text{Body he ght (cm)} \quad 100}$$

After the operat on the subjects lose we ght accord ng to a common pattern (1?) The ma n we ght loss occurs w th n the f rst postoperat ve year (period II F g ?) The rate of we ght loss s then constant and may be cal ula ed for each subject from the general exp ess on $Y \quad a{-}bx$ where Y s the body we ght ndex x the t me in weeks and b the change n body we ght ndex per week The correlat on coeff c ent fo th s relat on exceeded 0 9? in all subjects

Chem cal meth ds Fast ng levels of S tryptophan we e determ ned by a spec f c fluorometric method (10) mod f ed accord ng to Lehmann (19) g v ng normal values between 36 and 80 µmol/l S tyro n was determ ned (6) and the poss ble presence of v am n B_6 def en y was evaluated (13) Routine procedures were used to de

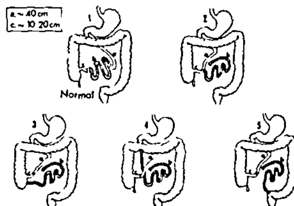


Fig 1 Types of anastomoses in small intestinal bypass operation

termine serum levels of albumin potassium calcium and magnesium

Serum levels of tryptophan The study comprised 52 subjects treated for obesity with jejunoileostomy Clinical data prior to the shunt operation are given in Table I

Fasting levels of S tryptophan were determined in 10 subjects prior to the operation Twenty-eight patients were examined during the first postoperative year In 9 of them the analysis was repeated during the second year together with sera from the remaining 24 patients A total of 126 blood samples were examined all drawn at 8 a m after an overnight fast A subject was included in the low tryptophan group if S tryptophan was below 36 μmol/l in two consecutive samples or in two out of three consecutive samples

Supplement study In a double blind cross-over trial 18 patients were given L tryptophan or placebo during periods of 14 days 10 started with tryptophan and switched over to placebo and 8 received the substances in the reverse order L tryptophan and placebo were administered in gelatine capsules (Astra Sweden) each containing 0 4 g and the patients were instructed to take 1 capsule three times daily before meals while on ordinary diet Fasting levels of S tryptophan were controlled in the morning before starting the medication and again on the 15th and 29th days approximately 14 hours after the last dosage

Ratings of psychopathology The 18 patients in the supplement study were rated on a recently developed comprehensive psychopathological rating scale CPRS (27)

Table I *Data on 52 patients with extreme obesity prior to jejunoileostomy*

	Mean±S E M	Range
Body height (cm)	170 1±0 9	155–185
Body weight (kg)	142 7±2 9	94–180
Age (y)	35 ±2	16– 59
Small intestinal length (m)	7 7±0 1	6 0–10 2

Table II *S tryptophan levels prior to and after jejunoileostomy*

Nine patients were examined during both the first and the second postoperative year 2 of them had low concentrations on both occasions

	Body weight index	No of pats	No of ana lyses	S tryptophan (μmol/l)
Preoperative	1 96±0 07	10	10	76 4±4 4
Postoperative				
1st y	1 29±0 04***	28	80	45 0±2 0
2nd y	1 26±0 04***	24+9	46	42 1±2 9 *

* * $p<0\ 001$

prior to treatment and immediately after each treatment period The ratings were based on a flexible psychiatric interview and performed by the same psychiatrist who was unaware of the S tryptophan levels Classification of the 18 patients in a low ($n=12$) and a normal ($n=6$) S tryptophan group was based on repeated previous analyses of S tryptophan and a control immediately prior to the interview

Statistical methods Mean values±S E M are given unless otherwise stated Significance tests were made according to Snedecor Non parametric methods were used for the analysis of rating data

RESULTS

Treatment of obesity with jejunoileal shunt was followed by a significant decrease ($p<0\ 001$) in fasting serum concentrations of S tryptophan (Table II)

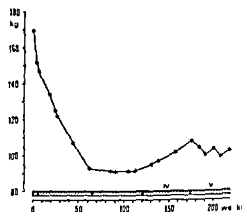


Fig 2 Weight kinetics after jejunoileostomy for treatment of obesity Period I postoperative catabolism with rapid weight loss Period II constant rate of weight loss Period III decelerating rate of weight loss Period IV slight weight increase Period V weight stabilization

Table III *Clinical data on 52 patients with postoperatively normal (>36 µmol/l) and low S tryptophan concentrations*

Min S tryptophan (µmol/l)	No of pats	Preoperative b wt index	Intraoperative small intestinal length (cm)	Intraoperative length of remaining small intestine (cm)	Postoperative constant weight loss (%/week)
43 5±1 9	23	1 99±0 05	771 5±22 6	60 4±1 3	0 91±0 08
21 7±1 6***	29	2 03±0 05	750 5±13 7	60 4±1 4	1 33±0 07***

*** $p<0.001$

which remained low throughout the first and second postoperative year

Preoperative serum levels were normal in all 52 patients 32 had a subnormal postoperative value on at least one occasion Based on repeated analyses 29 of the latter were considered as having permanently decreased serum levels their average minimum concentration being 21 7±1 6 µmol/l as opposed to 43 5±1 9 µmol/l in those 23 who were considered to have normal levels ($p<0.001$) In 10 patients the serum concentrations were less than half of the lower normal borderline level

Comparing the patients with low and normal serum tryptophan concentrations (Table III) no differences existed as to preoperative body weight index intraoperatively measured intestinal length surgical procedure performed or length of the postoperative intestinal segment in function The low tryptophan group had a significantly higher rate of weight loss (Table III) and a higher incidence of some postoperative complications (Table IV) than the normal tryptophan group

A positive correlation ($p<0.001$) existed between the serum concentrations of albumin and tryptophan (Fig 3) S tyrosine was not significantly altered by the surgical procedure and postoperative vitamin B_6 deficiency was not found

Oral L tryptophan supplement significantly increased the serum levels in 18 treated patients from 29 3±1 3 to 37 7±3 7 µmol/l ($p<0.02$) The dose was however insufficient to normalize low S tryptophan levels in only two out of 12 patients with an initially low concentration did the post treatment fasting serum concentration exceed 36 µmol/l

The median rating score for the total psychopathology on the CPRS was 8 5 (range 0–38) The normal range for psychiatrically healthy volunteers rated on the CPRS is 0–2 (28) Thirteen of the 18 patients had moderate or severe psychiatric disturbances Two patients had psychotic symptoms (hallucinations paranoid ideas) one of them had to be referred for further psychiatric treatment A large number of neurotic symptoms were also present (depressive mood anxiety phobias) Other common complaints were failing memory (2 in the normal and 5 in the low tryptophan group) and increased feelings of hostility (5 in the normal and 6 in the low tryptophan group) Although the median total score as well as the median subscale scores on anxiety and depression were higher in the low than in the normal tryptophan group none of the differences were significant The rating scores were not significantly altered by the daily intake of 1 2 g L tryptophan during a fortnight (Table V)

DISCUSSION

Small intestinal bypass surgery for treatment of incapacitating obesity implies the induction of a malabsorptive state by reducing the length of the small intestine in function to about 1/10 of its origi

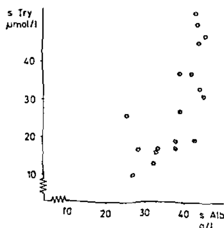


Fig 3 Relation between serum concentrations of tryptophan and albumin in 18 patients after intestinal bypass

Table IV *Complications following surgery with jejunoileal shunt in 52 obese subjects*

ED=electrolyte disturbances LI=signs of liver injury UTC=urinary calculi IM=immune reactions

Min S tryptophan (μmol/l)	No of pats	No of pats with complications			
		ED	LI	UTC	IM
43 5±1 9	23	1	0	3	4
21 7±1 6**	29	9*	8*	8	7

** $p<0.05$

nal length and is followed by a period of weight loss for about one year after which the body weight stabilizes at a new level (12) Postoperative changes of the amino acid pattern in plasma have been recorded both with increased (taurin and glutamatic acid) and decreased (leucine and other branched amino acids) concentrations of single amino acids (3) In the present study interest focused on the essential amino acid tryptophan low levels being found in more than half of a series of patients treated with jejunoileostomy The patients with low serum tryptophan levels had a more rapid weight loss and more complications than those with normal levels

It is reasonable to assume that the low serum levels resulted from a deficient intestinal uptake of tryptophan At least two conditions may have contributed to a deficient tryptophan absorption One is the shortness of the intestine in function Since the perfusion studies of Adibi and Gray (1) we know that the absorption of L tryptophan in a test segment when given in an equi molar amino acid solution or in proportion to its concentration in ordinary food is lower than that of other amino acids This means that a longer intestine or a longer transit time (14), is needed for its complete absorption From the same work it is known that increasing the relative amount of one amino acid in the perfusate promotes its absorption Thus if the shortness of the bowel is responsible one would expect to increase the serum levels by increasing the concentration of tryptophan in food or by giving tryptophan with the meals The finding of an increasing level of S tryptophan in 18 patients during supplementation with a moderate oral dosage of L tryptophan supports this reasoning Another mechanism could be the presence of an abnormal bacterial flora in the short intestine in some patients The bacteria may be either tryptophan consumers and hence decrease the amount available for absorption or producers of the enzyme tryptophanase in which case tryptyophan is degraded before its absorption Estimation of the urinary tryptophan metabolites is essential for evaluating the importance of this mechanism

There is a large body of evidence relating depressive illness to an alteration of serotonergic functions in the cerebral nervous system (5 6 18 24, 29) A decreased supply of the precursor amino acid tryptophan is one of several possible explanations Thus Lehmann has related psychiatric symptoms and low levels of S tryptophan in patients with the carcinoid syndrome (20) and an uncommonly high incidence of depressions was noted in patients with regional enteritis and low S tryptophan levels (15) Among the present patients symptoms of depression and anxiety were more common in the low than in the normal S tryptophan group but the difference was not significant The high overall incidence of psychopathology in our patients is in agreement with previous studies on patients with incapacitating obesity (12)

The main part of S tryptophan is bound to albumin It has been assumed that only the lesser free tryptophan fraction can pass over to the central nervous system and that a reduction of the free fraction might be important for the development of depressive symptoms In agreement is the finding by Coppen et al (7) of low levels of non protein bound tryptophan in depressive patients with normal S tryptophan levels

In the malabsorptive state after jejunoileostomy the free tryptophan fraction can be interfered with by concomitant changes of lipid metabolism (7) and by a lowered S albumin The appearance of the relation between S albumin and S tryptophan in the

Table V *Psychopathological rating scores (medians) in 18 patients with low (L n=12) or normal (N n=6) plasma levels of tryptophan before and after supplementation with 1 2 g L tryptophan daily during 2 weeks*

Rating scores	Before		After	
	L	N	L	N
Total psychopathology	8 50	7 25	10 00	5 50
Anxiety subscale	2 75	0 25	3 50	0 25
Depression subscale	6 25	4 50	7 25	3.50

present series is not congruent with a constant proportion of free to bound tryptophan at all albumin concentrations We therefore tried to separate the free tryptophan fractions which might correlate better to the rating scores of psychopathology in our patient sera but the conventional methods used (21 25) were too unreliable

Treatment with L tryptophan has been tried in depressive patients with normal S tryptophan levels since the report by Oates and Sjoerdsma (22) usually in a high daily dosage (8 9) In a previous uncontrolled pilot study on patients with inflammatory bowel disease (15) a daily substitution with 0 6–1 2 g L tryptophan was sufficient to normalize low serum levels and give relief of depressive symptoms

Although in the present series a daily extra intake of 1 2 g L tryptophan for two weeks significantly increased the serum levels they were not normalized in subjects with low pretreatment levels The effect of treatment on mental symptoms could therefore not be evaluated adequately

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Kaliuretic Effect of L-dopa Treatment in Parkinsonian Patients

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ABSTRACT Hypokalemia, sometimes severe, was observed in some L dopa treated parkinsonian patients. The influence of L dopa on the renal excretion of potassium was studied in 3 patients with hypokalemia and in 5 normokalemic patients by determination of renal plasma flow, glomerular filtration rate, plasma concentration of potassium and sodium as well as urinary excretion of potassium, sodium and aldosterone. L Dopa intake was found to cause an increased excretion of potassium and sometimes also of sodium in the hypokalemic but not in the normokalemic patients. This effect on the renal function could be prohibited by the administration of a peripheral dopa decarboxylase inhibitor. It is not known why this effect occurred in some individuals but not in others, but our results indicate a correlation between aldosterone production and this renal effect of L dopa.

Dopamine given intravenously has been reported to influence renal blood flow, glomerular filtration rate and urinary sodium excretion both in healthy individuals (14) and in patients with congestive heart failure (8). According to Finlay et al. (6) similar effects on renal function are induced by orally given L dopa. In a group of 250 parkinsonian patients we observed that the serum potassium concentration decreased in some of them during L dopa treatment. In about 10% of the group hypokalemia, i.e. values below 3.6 mmol/l, has been observed. Furthermore, some patients reported that L dopa intermittently caused increased diuresis.

Registration of diuresis and the urinary excretion of potassium and sodium was performed in one hypokalemic patient with such marked diuretic episodes. Furthermore renal plasma flow, glomerular filtration rate, plasma concentration of potassium and sodium, as well as urinary excretion of potassium, sodium and aldosterone were determined during standardized conditions in eight L dopa treated patients. Three of the patients had hypokalemia, one of them being the patient with marked variations in the diuresis, and five patients had normal serum potassium levels. These investigations were performed both with L-dopa alone and in combination with a peripheral dopa decarboxylase inhibitor.

MATERIAL

The patient material consisted of eight L-dopa treated parkinsonian patients (Table I), three of them with hypokalemia and five with normal serum potassium levels during L-dopa treatment. The normokalemic and the hypokalemic patients were matched with each other concerning the severity of the parkinsonian symptom and the improvement during L-dopa treatment, evaluated with a technique described earlier (9). As seen in Table I, patients 1 and 2 both had a low serum potassium concentration (reference value 3.6–5.1 mmol/l) before L-dopa treatment. The concentration, however, became normal when extra potassium was given orally. Furthermore, patient 1 reported a marked increase in the diuresis shortly after some of the dopa doses, lasting for about one hour and occurring several times a week. According to the patient this effect on diuresis was accompanied by a good effect of the L-dopa on the parkinsonian symptoms.

None of the eight patients were treated with any other drug known to influence the potassium metabolism, neither did they have edema or signs of liver or cardiac disease. Patient 3 was rather psycholabile and had a fluctuating BP but neither she nor any other patient had

Table I *Clinical data on eight parkinsonian patients*

Pat no	Sex	Age (y)	Duration of disease (y)	Duration of L-dopa treatment (mo)	L dopa dose g/d	L dopa dose g/kg b wt	Plasma concentration of potassium* (mmol/l) Before L-dopa No extra K	Before L-dopa With extra K	During L-dopa No extra K	During L-dopa With extra K
Hypokalemic										
1	♂	64	15	53	5 0	0 07	3 2	3 8	2 2	3 3
2	♂	67	6	27	3 2	0 05	3 0	3 7	3 2	3 3
3	♀	60	8	34	4 5	0 07	4 0	–	3 2	4 0
Mean		64	10	38	4 2	0 07				
Normokalemic										
4	♂	63	16	58	5 0	0 08	4 1	–	4 1	–
5	♂	56	17	60	2 6	0 03	4 2	–	4 0	–
6	♂	61	9	50	3 6	0 05	4 0	–	3 9	–
7	♀	65	6	48	1 8	0 04	4 3	–	4 3	–
8	♀	62	14	51	3 5	0 07	4 5	–	4 4	–
Mean		61	12	53	3 5	0 05				

* Means of several registrations during each period of treatment

any signs of systemic hypertension although the three hypokalemic patients had a higher BP (Table IV) than the normokalemic

METHODS

Initial study of the L-dopa effect on the diuresis in patient I

During a six-day period the diuresis and the excretion of potassium and sodium were measured on each day between 6 a m and 1 p m At 8 30 a m 1 g of L-dopa was given together with 100 ml of water and two pieces of bread and butter No further drug food or water was given from 10 p m on the day before the study until 1 p m on the day of the study The amount of urinary sodium excreted 30–180 min after intake of L-dopa was correlated to the plasma concentration of L-dopa at 60 min after L-dopa intake

Clearance studies

For four days before the clearance studies all eight patients kept to a controlled diet containing 50–70 mmol potassium and 150–180 mmol sodium per day and no further potassium or sodium was given

Inulin and para aminohippurate (PAH) clearances and the urinary excretion of sodium and potassium were determined twice in each patient first during their ordinary L-dopa treatment and later after treatment for more than ten days with a fixed combination of L-dopa and dopa decarboxylase inhibitor one tablet containing 250 mg of L-dopa and 25 mg of inhibitor (Sinemet® Merck Sharp & Dohme) The decarboxylase inhibitor L methyldopa hydrazine (Carbidopa) is reported not to pass the blood brain barrier (13 15 19) and therefore inhibits the production of dopamine from L-dopa only in the peripheral organs

The clearance studies were performed in the morning before food intake and after 12 hours withdrawal of L-dopa and other drugs In order to increase the diuresis a total volume of 1 5–2 0 l water was given during the clearance studies Each study included 6 clearance periods of 30 min and simultaneous determination of inulin and PAH clearance The urine was voided spontaneously The serum and urine concentrations of sodium and potassium were measured in all periods The urinary excretion of sodium and potassium was calculated as a percentage of filtered load In the middle of period 3 in the first study L-dopa was given in doses of 0 5 g (patients 3 5 7 8) or 1 g (patients 1 2 4 6) i e one or two tablets of Larodopa® and in the second study patients 3 5 7 and 8 received half a tablet and patients 1 2 4 and 6 one tablet of Sinemet® The clearance technique has been described in detail by Aurell et al (1) Dopa in serum was determined by ion exchange chromatography (2)

Aldosterone excretion was measured in the three hypokalemic and four of the normokalemic patients Urine was collected during 24 hours on the day before the clearance studies Aldosterone was determined densitometrically at 380 nm (16) after purification by thin layer chromatography (12) The procedure included hydrolysis extraction and thin layer chromatography in three different systems Furthermore the aldosterone fraction was oxydized by iodate before the second chromatographic run ^{14}C aldosterone added to the original urine was used as a marker for calculation of the loss during preparation

The plasma renin determinations were performed by M Aurell Department of Clinical Physiology University of Gothenburg

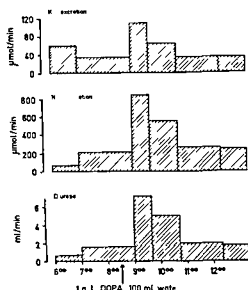


Fig 1 Change in potassium and sodium excretion and in the diuresis after 1 g L-dopa in patient 1

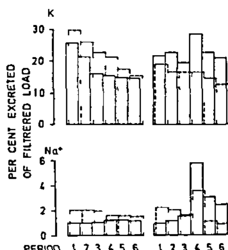


Fig 2 Urinary excretion of potassium and sodium during six clearance periods in two patients no 6 (normokalemic) (to the left) and no 2 (hypokalemic) (to the right) L-dopa was given in the middle of period 3 □=without inhibitor ⌐=with inhibitor

RESULTS

Initial study of the L dopa effect on the diuresis in patient 1

The osmolality of the morning urine specimens varied between 550 and 850 mosm/kg The increase in the diuresis and the plasma dopa concentration 60 min after L-dopa intake varied considerably between the different days There was no correlation either between these two parameters or between the urinary sodium excretion and the plasma dopa concentration On one of the days a very marked increase in the diuresis to a maximum of more than 7 ml/min was seen during 30–70 min after L-dopa intake (Fig 1) despite that fluid intake for the last 11 hours had been limited to 100 ml of water together with the L dopa tablets The sodium excretion increased parallel with the change in the diuresis to a maximum of 820 µmol/min The potassium excretion increased to a maximum of 110 µmol/min

Clearance studies

When clearance studies are performed in a post absorbtive state there is normally a successive decrease in the urinary potassium excretion (compare Fig 2) but an almost constant excretion of sodium The serum electrolyte levels are not significantly changed

Effect of L dopa In the five normokalemic patients the mean urinary excretion of potassium expressed as a percentage of the filtered load was 18 9% during the first three periods i e before L-dopa and 14 3% during the last three periods i e after L-dopa (Table II) The mean urinary excretion of sodium was 1 0% before and 1 1% after L-dopa administration (Table III) Thus the urinary excretion of both potassium and sodium in these patients was similar to that ordinarily seen in a postabsorbtive state

On the other hand in all three patients with hypokalemia L-dopa caused an increase in potassium excretion (Table II Fig 2) and in patient 2 an increase also in the sodium excretion (Table III Fig 2) The very marked increase in sodium excretion observed in patient 1 at the initial diuresis study did not occur at the time of the clearance study However even before L dopa administration at the clearance study the urinary sodium loss (Table III) was high in this patient compared with the others

There appeared to be a slight increase in PAH clearance when L-dopa was given but there was no obvious difference in this respect between the hypokalemic and the normokalemic patients (Table IV)

Effect of L dopa+inhibitor In the five normokalemic patients the mean urinary excretion of

Table II *Urinary excretion and plasma concentration of potassium in L-dopa treated parkinsonian patients before (period 1–3) and after (period 4–6) administration of L dopa without and with decarboxylase inhibitor*

Pat no	Without inhibitor: Plasma concentration (mmol/l) 1–3	4–6	Urinary excretion: % of filtered load 1–3	4–6	µmol/min 1–3	4–6	With inhibitor: Plasma concentration (mmol/l) 1–3	4–6	Urinary excretion: % of filtered load 1–3	4–6	µmol/min 1–3	4–6
Hypokalemic												
1	2 2	2 2	38 5	41 7	52	59	3 4	3 4	29 6	21 9	83	48
2	3 2	3 3	17 4	24 2	42	63	3 4	3 5	21 5	15 2	53	41
3	3 3	3 2	12 9	13 4	35	38	3 3	3 4	11 9	14 1	30	39
Mean	2 9	2 9	22 9	26 4	43	53	3 4	3 4	21 0	17 1	55	43
Normokalemic												
4	4 1	3 9	18 9	15 0	58	42	4 1	4 1	32 2	21 8	86	[illegible]
5	4 3	4 0	20 6	14 7	95	57	4 6	4 5	14 9	13 1	59	62
6	3 7	3 5	21 6	15 9	63	42	4 0	3 8	26 0	18 2	84	44
7	3 7	3 6	15 3	13 4	45	44	3 9	3 6	15 8	10 2	56	35
8	4 2	3 8	18 3	12 7	56	36	3 8	3 8	25 3	18 1	51	70
Mean	*4 0*	*3 8*	*18 9*	*14 3*	*63*	*44*	*4 1*	*4 0*	*22 8*	*16 3*	*67*	*59*

Table III *Urinary excretion and plasma concentration of sodium in L dopa treated parkinsonian patients before (period 1–3) and after (period 4–6) administration of L dopa without and with decarboxylase inhibitor*

Pat no	Without inhibitor: Plasma concentration (mmol/l) 1–3	4–6	Urinary excretion: % of filtered load 1–3	4–6	µmol/min 1–3	4–6	With inhibitor: Plasma concentration (mmol/l) 1–3	4–6	Urinary excretion: % of filtered load 1–3	4–6	µmol/min 1–3	4–6
Hypokalemic												
1	141	145	3 9	3 2	343	268	137	136	2 5	1 7	278	148
2	139	139	1 2	3 7	119	412	136	134	1 9	1 8	186	136
3	140	140	0 5	0 5	60	73	138	137	1 0	1 1	115	[illegible]
Mean	140	141	1 9	2 5	174	251	137	136	1 8	1 5	193	135
Normokalemic												
4	138	142	1 1	1 4	111	149	139	140	1 5	1 5	146	196
5	137	137	0 8	1 0	113	139	141	142	0 5	0 8	58	119
6	136	134	1 0	1 2	117	115	136	136	2 0	1 6	222	143
7	141	141	0 8	0 8	86	110	138	138	0 6	0 6	88	83
8	138	138	1 1	1 2	118	139	134	136	0 7	0 9	55	174
Mean	138	138	1 0	1 1	109	130	138	138	1 1	1 1	114	133

potassium was 22 8% before and 16 3% after the drug had been given i e as on L-dopa alone the excretion of potassium decreased successively (Table II Fig 2)

Similar results for potassium excretion were now found in two patients with hypokalemia (nos 1 and 2) in whom the excretion decreased from 29 6% to 21 9% and from 21 5% to 15 2% respectively (Table II Fig 2) In these two patients the addition of the inhibitor to L dopa thus seemed to abolish the

Table IV *Blood pressure aldosterone excretion in urine inulin (C_{In}) and PAH clearances (C_{PAH}) in eight parkinsonian patients receiving L dopa without and with decarboxylase inhibitor*

Pat no	Without inhibitor BP[a] (mmHg)	Aldosterone[b] (nmol/24 h)	C_{I}[c] (ml/min) 1–3	C_{I}[c] (ml/min) 4–6	C_{PAH}[c] (ml/min) 1–3	C_{PAH}[c] (ml/min) 4–6	With inhibitor BP[a] (mmHg)	Aldosterone[b] (nmol/24 h)	C_{In}[c] (ml/min) 1–3	C_{In}[c] (ml/min) 4–6	C_{PAH}[c] (ml/min) 1–3	C_{PAH}[c] (ml/min) 4–6
Hypokalemic												
1	173/110	69	62	60	327	352	168/120	100	76	62	409	354
2	158/105	47	75	80	348	365	170/98	52	73	78	374	430
3	197/115	33	83	88	420	432	173/108	31	76	83	415	448
Mean	176/110	50	73	76	365	383	170/109	56	75	74	399	411
Normokalemic												
4	142/92	50	76	74	421	436	138/92	36	73	95	415	507
5	110/80	–	107	97	630	603	119/79	–	85	107	492	727
6	142/98	17	83	76	396	357	153/97	15	82	68	485	399
7	150/87	22	79	89	371	423	135/75	19	91	94	396	449
8	140/98	15	75	76	406	481	150/98	21	60	103	364	583
Mean	137/91	26	84	82	445	460	139/88	23	78	93	430	533

[a] Means of four values measured on four consecutive mornings before any drug was given
[b] Measured on the day before the clearance studies
Determined before (period 1–3) and after (period 4–6) administration of L-dopa

effect on potassium excretion caused by L dopa alone In patient 3 however there was still a slight increase in the excretion of potassium when L dopa was given together with the inhibitor In patient 2 who had a markedly increased sodium excretion after L dopa intake no such obvious effect was seen when the inhibitor was added (Table III Fig 2)

When L dopa was given together with the peripheral inhibitor there also appeared to be a slight increase in the PAH clearance as on L dopa alone but no difference was found between the hypokalemic and normokalemic patients (Table IV) In patient 1 with a very low plasma potassium level initially the concentration increased during the period of combined treatment but in the other six patients the levels did not change (Table II)

Aldosterone analyses

According to experience in our hospital the aldosterone excretion in primary aldosteronism mostly exceeds 60 nmol/day although the diagnosis cannot be excluded until the excretion is below 30 nmol/day In the present study all the three hypokalemic patients had values above 30 nmol/day and patient 1 excreted more than 60 nmol/day (Table IV) Three of the normokalemic patients excreted less than 30 and one between 30 and 60 nmol/day The amount of aldosterone excreted was of the same magnitude whether L-dopa was given alone or together with the peripheral decarboxylase inhibitor

Additional studies in patient 1

Addition of 10 g (170 nmol) sodium chloride per day to the food for three weeks did not influence either the serum potassium level or the excretion of aldosterone the latter being 72 94 and 111 nmol/day respectively at the end of this three week period The basal plasma renin activity was low i e 0 2 μg angiotensin/ml/hour (reference values 0 3–2 0) (7) After one week of treatment with an aldosterone antagonist spironolactone (Aldactone® 25 mg four times a day) together with 80 mmol potassium in tablets normal serum potassium values (4 2–4 4 mmol) were found for the first time since hypokalemia had developed during L-dopa treatment When the potassium tablets were withdrawn and the treatment with spironolactone was unchanged the serum potassium level was 3 8–4 1 mmol/l The urinary excretion of aldosterone was 75 92 83 70 and 49 nmol/day during the 17 days of spironolactone administration

DISCUSSION

We have observed a decreased serum potassium concentration and an increased kaliuresis in some parkinsonian patients during L-dopa treatment Furthermore certain of these patients occasionally had a very marked diuretic effect of L-dopa The present studies show that L dopa was responsible for these renal effects We have no definitive explanation for these observations but some hypotheses will be offered

The urinary excretion of potassium and sodium was markedly increased in some patients shortly after intake of L dopa This effect which could be prohibited by addition of a dopa decarboxylase inhibitor was seen only in patients who had developed hypokalemia during L dopa treatment indicating that their hypokalemia was caused by this increased potassium excretion

A common feature of the patients developing hypokalemia during L dopa treatment seemed to be that they had higher urinary aldosterone values than the normokalemic patients One of the hypokalemic patients no 1 most probably had a primary aldosteronism The kaliuresis the hypokalemia the somewhat elevated BP the high values of aldosterone in combination with the low plasma renin activity and the good effect of spironolactone on serum potassium level speak in favour of this osis However the serum potassium level was in normal limits before L dopa treatment when a potassium was added During L-dopa treat ient a normal serum potassium level was no longer achieved even when very high doses of potassium were given The clear correlation in time between intake of L-dopa and the kaliuresis and the observation that this kaliuretic effect of L dopa was abolished when a dopa decarboxylase inhibitor was added indicate that the L dopa therapy most probably contributed to the hypokalemia and that thus a primary aldosteronism was not the sole explanation of the low plasma potassium level The aldosterone excretion in the other two hypokalemic patients was not of the same magnitude as in patient 1 assumed to have primary aldosteronism but still higher than in most of the normokalemic patients Both of these patients reacted to L dopa with kaliuresis but the addition of a dopa decarboxylase inhibitor abolished this renal influence of L-dopa in only one of them (no 2) *Such an individual variation* in the effect of the inhibitor might be due to a difference in its efficacy in different organs It is not clear why these two hypokalemic patients had a somewhat high aldosterone production but a primarily high production might be the reason In none of the patients did the clinical examination reveal symptoms of diseases causing secondary aldosteronism

The possibility of a secondary aldosteronism caused by the L-dopa treatment must also be considered As the advanced parkinsonian symptoms contraindicated withdrawal of L-dopa the aldosterone determinations were made only when the patients were on L-dopa treatment with and without a concomitant inhibitor Theoretically the aldosterone production could be influenced by dopamine either by a peripheral renal or a central cerebral effect Renal stimulation of aldosterone production is known to be caused by e g loss of sodium and reduction of the extracellular water (4 5) and as dopamine causes natriuresis (6 8 14) this seemingly could be the explanation for the increased aldosterone production However the addition of the decarboxylase inhibitor decreasing the amount of dopamine in the peripheral blood did not seem to decrease the aldosterone level which contradicts the possibility of the increased aldosterone production being caused by a peripheral dopaminergic effect

Regarding a cerebral dopaminergic effect it is known that L dopa influences hypophyseal hormone production such as growth hormone (3) and prolactin (10 18) The aldosterone production has been shown to be influenced by corticotropin but this stimulation seems to be only transient and furthermore followed by a decreased production of aldosterone (11 15 17) The possibility of an increased aldosterone production caused by a dopaminergic influence on hypophyseal hormones still seems to be of merely hypothetical interest

Our conclusion is thus that L dopa treatment in some parkinsonian patients increases urinary excretion of potassium causing hypokalemia which is of practical clinical interest It is not known why this effect appears in some individuals but not in others Our results indicate a correlation between the aldosterone production and this renal effect of L dopa

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Lymphocytic Thyroiditis

I Correlation between Morphological Immunological and Clinical Findings

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ABSTRACT Biopsies from the thyroid glands in 32 selected patients with goitre and lymphocytic thyroiditis have been investigated with quantitation of the morphological changes This permitted a comparison with immunological and clinical findings The three main elements in the destruction of glandular tissue—lymphocytes, plasma cells and fibrosis—varied relatively independently of each other as an expression of the great variation in the appearance of the tissue lesion Thyroglobulin antibodies showed a correlation to the number of plasma cells whereas the microsomal thyroid antibodies showed a correlation to the number of lymphocytes The morphologic changes were independent of the duration of the disease The degree of fibrosis increased parallel with age and there was a tendency towards glandular fibrosis in myxoedematous patients

When Hashimoto in 1912 described 4 cases of struma lymphomatosa (8) he emphasized the lymphocytic infiltration with follicles in the enlarged thyroid gland Since then the terms lymphocytic thyroiditis or autoimmune thyroiditis have been applied to conditions which by histological or clinical means have been designated as asymptomatic thyroiditis focal thyroiditis atrophic thyroiditis lymphocytic thyroiditis with thyrotoxicosis and Hashimoto s thyroiditis Some investigators found a common pathophysiological entity of these diseases whereas others made subdivisions constituting separate disease entities

In order to avoid confusion between non identical disease entities the present paper is confined to one only namely *simultaneous occurrence of goitre and lymphocytic thyroiditis without thyrotoxicosis* A semiquantitative method was used for investigating the morphological findings thereby permitting a comparison with immunological and clinical data

MATERIAL

In a series of 45 patients with lymphocytic thyroiditis verified histologically by open biopsy only 32 fulfilled the criterion mentioned above The patients were 27 women and 5 men with an age range of 18–86 years (average 50) and duration of the disease of 1 month–24 years (average 39 months) The duration of the disease is identical with the duration of goitre Twenty four patients were euthyroid and 8 suffered from myxoedema according to clinical and laboratory investigations

METHODS

The tissue amounting to 0 5–10 g was excised by open surgical biopsy Formalin fixation (10%) and normal paraffine embedding technique were used The sections were stained with hematoxylin-eosin van Gieson s collagen stain reticulin stain Unna Pappenheim s methyl green pyronine method periodic acid Schiff method (PAS) and Toluidine blue

The sections were evaluated by three of us (H E C J S I R) independently and without knowledge of the clinical data The presence of PAS positive material number of lymphocytes and plasma cells as well as the degree of fibrosis and amount of reticulin fibres were estimated semiquantitatively as an average of the results of the three investigators The occurrence of epithelial changes with metaplasia to Askanazy cells was noted but not graded Generally there was a rather astonishing agreement and revision was necessary in only a few cases as opinions differed about some of the morphological features

Thyroglobulin antibodies were estimated by passive haemagglutination test with conventional technique and thyroid microsomal antibodies by a complement fixation test

Table I *Correlation between morphological variables*

	Lymphocytes	Plasma cells	Reticulin	Fibrosis
Lymphocytes	–			
Plasma cells	0 0204	–		
Reticulin	0 1015	0 3737*	–	
Fibrosis	0 3562*	0 2500	0 4598***	–
PAS	−0 3726*	−0 5022***	−0 4200**	−0 5066***

Spearman s rank correlation coefficient
$N=32$ * $p<0.05$ ** $p<0.02$ *** $p<0.01$

The statistical evaluations have been made with the χ^2 test with Yate s correction Mann Whitney s rank sum test for unpaired data and Spearman s rank correlation coefficient (16)

RESULTS

Table I shows the correlations between the semi quantitative morphological variables as estimated by Spearman s non parametric rank correlation coefficient PAS staining proved to be the best method for evaluating the degree of destruction of glandular tissue as the amount of PAS positive material (colloid) was inversely correlated to the cell infiltration as well as to the degree of fibrosis and reticulin content The extent of fibrosis naturally correlated well with the content of reticulin but was independent of plasma cell infiltration However the degree of fibrosis showed a weak correlation to the number of lymphocytes The number of lymphocytes and of plasma cells were mutually independent The three main features of the tissue change—lymphocytes plasma cells and fibrosis—were principally independent of each other

Table II shows the correlation between thyroid antibodies and the morphological features There were 22 patients (69%) with thyroglobulin antibodies and 17 (53%) with microsomal antibodies 13 patients (41%) exhibited both kinds of antibodies in their sera but 6 patients (19%) showed neither kind at the time of investigation

Table II *Correlation between morphological findings and thyroid antibodies*

	Thyroglobulin antibodies (22 pats)	Microsomal antibodies (17 pats)
Lymphocytes	0 0875	0 4607***
Plasma cells	0 5018* *	0 3570*
Fibrosis	0 2687	0 1933
PAS	−0 7356***	−0 3990**

Spearman s rank correlation coefficient
$N=32$ * $p<0.05$ ** $p<0.025$ *** $p<0.01$

Both thyroglobulin and microsomal antibodies were well correlated to the degree of glandular tissue destruction as estimated by the PAS staining procedure The thyroglobulin antibodies correlated mainly to the number of plasma cells whereas the microsomal antibodies did so chiefly to the number of lymphocytes and only to a lesser degree to the occurrence of plasma cells The strength of antibody titres did not show any correlation to the extent of fibrosis

Morphological and clinical findings

Age as such showed a correlation to the degree of fibrosis For instance the three degrees of fibrosis —*slight moderate and severe—were* associated with average ages of 43 55 and 57 years respectively The difference between the first and the last two degrees is significant ($p<0.05$) by the Mann Whitney test When allowance is made for the duration of the disease the results for age at onset are 40 51 and 54 years in the three severity groups i e the same pattern Age turned out to be independent both of the total severity of glandular lesion as estimated by PAS staining and of the number of lymphocytes and plasma cells In addition no correlation was found between age and the presence or strength of thyroid antibody titres

For technical reasons thyroid stimulating hormone was not estimated at the time of biopsy There was no significant difference in the morphological picture even a tendency towards more pronounced glandular lesion and more pronounced fibrosis in myxoedematous patients Thyroid function was not correlated to the presence or number of thyroid antibodies

DISCUSSION

Applying statistical calculations to the evaluation criteria renders the material extremely homogeneous

Quantitation of morphological changes in lymphocytic thyroiditis has seldom been mentioned in the literature (6 11) The destruction of glandular tissue in the thyroids is defined by the degree of epithelial cell destruction and/or metaplasia the degree of lymphocyte and plasma cell accumulation and the degree of fibrosis In the present material however the best parameter for this purpose turned out to be the PAS staining

The number of lymphocytes and plasma cells varied independently a phenomenon which is of importance for the relationship between cell infiltration and the presence of thyroid antibodies A weak positive correlation was found here between the number of lymphocytes and the degree of fibrosis but the importance of this seems to be rather uncertain in view of the slight changes which occur in the glandular tissue over several years (18) The relative independence of the three main features—lymphocytes plasma cells and fibrosis—in the glandular tissue destruction may reflect a pathophysiological inhomogeneity or it may express the variations occurring in tissue lesions which give an identical clinical picture

Largely varying frequencies of thyroid antibodies present in human lymphocytic thyroiditis have been reported (1 5 10 11 13 14) Part of these variations may be explained by differences in techniques and morphological criteria The present findings display a similar variation

Opinions differ as to whether or not the glandular lesion is reflected by the antibody titres Some authors (6 11 12 17) found no such relationship others (2 3 4 7 9 13 15) report varying degrees of correlation In the present series there was a good correlation between total glandular destruction and antibody titres Thyroglobulin antibodies and to a lesser degree the microsomal antibodies correlated with the number of plasma cells which may correspond to the role of these cells in the production of humoral antibodies Furthermore a significant correlation was observed between the number of lymphocytes and microsomal antibodies This finding may indicate that microsomal antigen acts as a stimulus for the growth of one or more subpopulations of lymphocytes and thereby for the induction of cell bound immune mechanisms or delayed hypersensitivity Wartenberg et al (19) found a relationship between T cell hypersensitivity and the microsomal antigen using the leucocyte migration inhibition test in patients with autoimmune thyroiditis

The duration of the disease did not correlate with the morphological changes This should indicate that the morphology does not change much during the course of the disease and is in accordance with the results of Vickery and Hamlin (18) These authors using repeated biopsies found a slightly progressive fibrosis in less than one half of their patients and no significant changes in the majority

The relationship between age and morphological alterations was especially investigated by Persson (11) He found increasing occurrence of Askanazy cells and plasma cells with increasing age This was not the case in our material On the contrary we found more fibrosis at higher ages This would seem natural if older age were equivalent to a longer duration of the disease but this is not the case The finding may therefore indicate that increasing age at onset involves a greater tendency to fibrosis The latter view is in accordance with that of Saxena and Crawford (14) who found very little fibrosis in juvenile lymphocytic thyroiditis

Some patients did develop myxoedema months to years after the time of biopsy If the morphology is more or less independent of the duration of the disease it might be assumed that the morphology at the time of biopsy could serve as a guide to the prognosis i e thyroid function Furthermore it might be assumed that a high age at onset with the increased tendency to fibrosis should mean an earlier development of myxoedema in this age group

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Effect of Long-term Vitamin D_2 Treatment on Bone Morphometry and Biochemical Values in Anticonvulsant Osteomalacia

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ABSTRACT Quantitative morphometric analyses of iliac crest biopsies from 20 epileptic patients receiving chronic anticonvulsant therapy have been performed before and after 4–8 months of vitamin D_2 treatment with 9000 U per day Biochemical quantities including serum 25 hydroxycholecalciferol (25 HCC) and serum parathyroid hormone (iPTH), were measured The anticonvulsant osteomalacia found in the initial bone biopsies was characterized by an increased amount of unmineralized bone, an increased bone resorption and contrary to vitamin D deficiency, an increased bone mineralization and bone formation Bone resorption and bone formation were probably equally increased since the amount of cancellous bone was normal Except for a slight increase in osteoid covered surfaces and osteoclastic resorption surfaces the bone changes were normalized after vitamin D_2 treatment leading to a mean serum level of 25 HCC 2 4 times above normal Serum iPTH was normal *before and unchanged during D_2 therapy The uri*nary calcium excretion remained decreased The investigation characterizes anticonvulsant osteomalacia as a specific bone disease different from that of vitamin D deficiency but induced by abnormalities in vitamin D metabolism or in the effects of vitamin D metabolites on receptor cells

Hypocalcemia elevated serum levels of alkaline phosphatase *decreased bone mineral content and* morphometric bone changes analogous to osteomalacia are frequent findings in patients receiving chronic anticonvulsant therapy (5 13 17) This drug induced osteomalacia is considered to stem from altered hepatic vitamin D metabolism (7 20) caused by induction of the hepatic P-450 microsomal enzyme activity In accordance with this low serum levels of 25 hydroxycholecalciferol (25 HCC) the hepatic vitamin D_3 metabolite have been reported in epileptic patients (2 8 12) The degree of osteomalacia is however not correlated to serum 25 HCC concentrations (12) and the bone changes in anticonvulsant osteomalacia differ from those seen in vitamin D deficiency in having increased surfaces covered with active osteoblasts in trabecular bone (19)

We have studied the effects of long term vitamin D_2 treatment on bone morphometry and mineralization activity and on biochemical quantities concerning calcium–phophorus metabolism in 20 epileptic patients with established bone changes in an attempt to clarify the nature of the bone disease and to assess the therapeutic value of vitamin D_2 supplements

PATIENTS

The study comprised 20 adult epileptic outpatients 10 men aged 21–39 years (mean 26 5) and 10 women aged 20–52 years (mean 31 5) All patients received diphenyl hydantoin some of them in combination with other anticonvulsant drugs and all had been treated for more *than 10 years The patients were selected from a group of* 60 epileptics in whom bone biopsy was performed (13) Entry into the trial was based on the occurrence of an increased amount of osteoid or an increase in the osteocytic osteolysis None of the patients had symptoms of bone disease and all had a normal serum creatinine value The

Table 1 *Chemical quantities in epileptic patients before (E(−D)) and after (E(+D)) vitamin D_2 treatment and in normal controls (C)*

	Corrected S-calcium (mmol/l)	S phosphorus (mmol/l)	S alkaline phosphatase (U/l)	S iPTH (pg/ml)	S 25 HCC (ng/ml)	U-calcium (mmol/mol creatinine)
E(−D) (N=20)						
x	2 483	1 26	198	61.8	17 5[a]	292
S E	0 017	0 04	11	7 1	2 0	22
E(+D) (N=20)						
x	2 507	1 18	165	59 3	67 7	303
S E	0 012	0 04	9	7 2	5 7	29
C (N=60)						
x	2 521	1 18	147	67 5[b]	28 2[c]	430
S E	0 009	0 02	4	2 2	2 2	13
p values						
E(−D)/E(+D)	=0 10	<0 05	<0 01	n s	<0 01	n s
E(−D)/C	<0 05	<0 01	<0 01	n s	<0 01	<0 01
E(+D)/C	n s	n s	<0 01	n s	<0 01	<0 01

[a] N=19 [b] N=76 [c] N=56

patients received an oral dose of 9000 U (0 23 mg) vitamin D_2 per day for 4–8 months without calcium supplements The anticonvulsant therapy was continued unchanged Both initial and control investigations were performed in the winter

METHODS

Bone biopsies were performed by transfixing the iliac crest after double labelling with tetracycline The following measurements were performed on decalcified and undecalcified bone sections (9 10) *absolute volume of trabecular bone* (AVTB) (% of total bone area) *osteoid surfaces* (OS) (% of total trabecular bone surfaces) *relative osteoid volume* (OV) (% of total trabecular bone volume) *mean width of osteoid seams* (WOS) (μm) as the mean of four extreme measurements in all surfaces covered with osteoid *trabecular osteoclastic resorption surfaces* (RS) (% of total trabecular bone surfaces) and *mean volume of periosteocytic lacunes* (POL) (μm³) as the mean of the product of length and width of 50 randomly selected lacunes Using ultraviolet light and undecalcified sections the mineralization activity was evaluated by measuring *calcification rate in trabecular bone* (CR) (μm/day) as the mean distance between the fluorescent tetracycline lines in all double labelled zones *active trabecular calcification surfaces* (ATCS) as the tetracycline labelled surfaces (% of total trabecular bone surfaces) and *active osteoid calcification surfaces* (AOCS) as tetracycline labelled surfaces (% of osteoid covered surfaces) AOCS is identical with *calcification fronts* measured by special staining techniques (16)

Serum 25 HCC was measured by a competitive protein binding assay (6) with a modification in the extraction procedure and the chromatographic step The coefficient of variation of repeated measurements at the level of 15 ng was 13 5% The sensitivity in the routine assay was 1.5 ng/ml

Serum immunoreactive parathyroid hormone (iPTH) was measured after extraction of the hormone from serum by adsorption to and elution from a microfine precipitated silica (Quso G 32) providing a hormone concentration 3 7 times higher in extract than in serum (4) Bovine PTH was used for ^{125}I labelling The coefficient of variation was 16% for measurements within the normal range (30-105 pg/ml bovine equivalents MRC bPTH standard 71/324) and the sensitivity 10 pg bPTH present in the incubation mixture

Serum concentrations of calcium phosphorus and alkaline phosphatase were measured by standard laboratory methods Serum calcium was corrected for individual variations in serum protein concentrations (15) The urinary 24 hour excretion of calcium and creatinine was determined on a non restricted diet The urinary calcium excretion was expressed as mmol calcium/mol creatinine excreted

Statistical significance of differences in group means

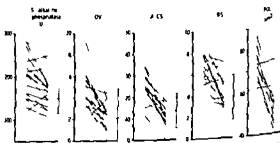


Fig 1 Serum alkaline phosphatase relative osteoid volume (*OV*) active trabecular calcification surfaces (*ATCS*) trabecular osteoclastic resorption surfaces (*RS*) and mean size of periosteocytic lacunes (*POL*) in epileptic patients before and after vitamin D_2 treatment. Mean+2 S D from normal controls are indicated

Table II *Bone morphometry in ep leptic patients before (E(−D)) and after (E(+D)) vitamin D_2 treatment and in normal controls (C)*

	AVTB (%)	OS (%)	OV (%)	WOS (μm)	RS (%)	POL (μm^2)	CR (μm/d)	ATCS (%)	AOCS (%)
E(D) (*N* 20)									
x	21 7	28 1	4 8	12 2	6 3	71 9	0 66	24 7	88 7
S E	1 1	1 4	0 4	0 4	0 3	1 9	0 02	1.2	2 8
E(+D) (*N*=19)									
x	21 0	21 8	2 2	8 9	4 8	53 1[b]	0 66	13 0	67 3
S E	1 3	1 6	0 3	0 7	0 4	1 7	0 0?	0 9	5 7
C (*N*=28)									
x	23 9	13 9	2 1	8 9[d]	3 8	52 0	0 70[f]	13 5[g]	86 6[e]
S E	1 1	1 3	0 3	0 3	0 2	0 7	0 03	1 3	5 7
p values									
E(D)/E(+D)	n s	<0 0?	<0 01	<0 01	<0 01	<0 01	n s	<0 01	<0 01
E(D)/C	n s	<0 01	<0 01	<0 01	<0 01	<0 01	n s	<0 01	n s
E(+D)/C	*n s*	*<0 01*	*n s*	*n s*	*<0 05*	*n s*	n s	*n s*	*<0 0?*

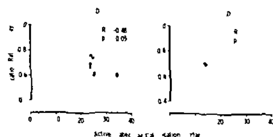
N 14 [b]*N* 20 *N* 18 [d]*N* 15 *N* ?0 [f]*N* 11 [g]*N* 10

was determined by Wilcoxon tests for two samples or for paired samples and correlat on coeff c ents by Spearman s rank correlation (*R*)

RESULTS

B ochemical values measured in the epilept c pa t ents before and after vitamin D_2 treatment and in age and sex matched normal controls are given in Table I together with the significance of differences *between the groups Before treatment the ep lept c* pat ents demonstrated a decrease in the mean serum calcium concentration and in the renal calcium excret on After v tam n D_2 treatment there *was an insign f cant rise in the mean serum calcium* concentrat on to a value which d d not differ from that of normal controls The renal calcium excre tion remained unchanged The in tially increased mean serum phosphorus level normalized *dur ng* vitam n D_2 treatment The mean serum alkaline phosphatase concentration decreased s gn f cantly during treatment but was still increased after 4–8 months of therapy (F g 1) The mean serum iPTH level was normal before and unchanged during vita m n D_2 treatment Serum ?5 HCC was in tially re duced During v tam n D_2 treatment serum 25 HCC increased to a mean concentrat on ? 4 t mes h gher than that of normal controls

Bone morphometry Table II gives the results of the morphometr c analyses of bone changes in ep leptic patients before and after vitam n D_2 treat *ment and in normal controls with the same age and* sex d str but on The most important changes in bone morphometry induced by vitam n D_2 are il lustrated in Fig 1 No difference was found be tween the groups in the amount of cancellous bone (AVTB) The initial increase in the amount of un calcified bone (OS OV WOS) was sign f cantly reduced by vitamin D_2 treatment but a sl ght in crease in OS rema ned The pretreatment increase in bone resorption (RS POL) was normal zed ex cept for a persistent slight elevat on in RS The *calc ficat on rate (CR) was insign f cantly decreased* before and unchanged during v tamin D treatment The percentage of trabecular bone surfaces active in bone mineral zation (ATCS) was increased be fore and normalized during vitam n D treatment An inverse correlat on was found between CR and ACTS before v tamin D_2 treatment (*R* 0 48 $p<0$ 05) but not after (*R* 0 14 $p>0$ 10) (Fig 2) The amount of osteo d *mineralized per day* esti mated as the product of CR and ATCS was init ally



F g 2 Correlat ons between calc f cat on rate and act e trabecular calc f cation surfaces n ep lept c pat ents be fore (*t D*) and after (+ *t D*) v tam n D_2 treatment

increased (16 3±0 9 ($\bar{x}$±S E)) compared with normal controls (9 7±1 2) ($p<0\ 01$) and decreased ($p<0\ 01$) during vitamin D_2 treatment to a mean value (8 6±0 6) which was not different from that of normal controls The percentage of osteoid covered surfaces active in mineralization (AOCS) was normal before and reduced after vitamin D_2 treatment

DISCUSSION

The present study demonstrates that the bone changes found in epileptic patients receiving long term anticonvulsant therapy are similar in some respects to those seen in vitamin D deficiency but different in others As in vitamin D deficiency (1 16 19 21) both the amount of unmineralized bone (OS OV WOS) and the resorptive activity in bone (RS POL) were increased Contrary to the findings in vitamin D deficiency (1 16 21) the active mineralization was increased as demonstrated by a two fold increase in the percentage of trabecular bone surfaces with active mineralization and a very small decrease in the calcification rate The increases in both unmineralized bone matrix (osteoid) and in mineralization activity indicate increased bone formation in anticonvulsant osteomalacia Contrary to vitamin D deficiency (1 3) anticonvulsant osteomalacia is therefore characterized by an increased bone turnover with a probably equal increase in bone formation and bone resorption rates as indicated by the normal amount of trabecular bone after more than 10 years of anticonvulsant treatment Futhermore the normal AOCS demonstrates that the mineralization of osteoid is normal in anticonvulsant osteomalacia

Biochemically anticonvulsant osteomalacia differs from vitamin D deficiency by an increased or normal (2 13) mean serum phosphorus concentration a normal (12) or only slightly elevated (2) mean serum level of iPTH and only a slight decrease in the mean serum 25 HCC level (2 8 12)

The abnormalities in bone and serum in anticonvulsant osteomalacia are however to a large extent normalized after pharmacological doses of vitamin D This indicates that the bone changes are induced by some alteration in vitamin D metabolism or in the effects of vitamin D metabolites on receptor cells and not by an unspecific effect of anticonvulsant drugs on bone cells

In the present study 4–8 months of vitamin D_2 treatment leading to a mean serum concentration of 25 HCC 2 4 times above normal did not change the reduced renal calcium excretion and slight changes in bone morphometry persisted This may be due to a considerable delay in the processes leading to normalization of bone changes However the bone mineral content in epileptic patients treated with 4 000 U vitamin D_2 per day was found to reach normal level after 3 ½ months of therapy (18) The results therefore, suggest a disturbance of vitamin D metabolism which is only partly overcome by increasing the mean serum level of 25-HCC This is supported by the observation that the biological action of both vitamin D_3 and 25 HCC is impaired in rachitic chicks treated with phenobarbital (14) and by the lack of correlation between serum 25 HCC and morphometric bone changes in 60 epileptic patients receiving chronic anticonvulsant therapy (12)

In the present study the mean serum concentration of iPTH was normal in the epileptic patients Increased serum levels of iPTH have been reported in a group of patients receiving chronic anticonvulsant therapy (2) However these patients demonstrated considerably more reduced serum levels of calcium and 25 HCC These observations are in agreement with the inverse correlation found between serum iPTH and serum 25 HCC in epileptic patients (2 12) The bone morphometry normalized during vitamin D_2 treatment without changes in the mean serum iPTH level, but the increase in serum calcium was small and insignificant The initial increase in POL which in primary hyperparathyroidism reflects PTH secretion (11) might be due to an increased cellular sensitivity to circulating PTH induced by the slight decrease in mean serum calcium concentration

The vitamin D_2 treatment induced a considerable reduction in the mean serum level of alkaline phosphatase The slightly elevated serum levels after 4–8 months of therapy may be due to the minimal bone changes still present or to an increase in liver isoenzymes caused by hepatic induction Serum alkaline phosphatase is however the most suitable biochemical quantitiy in the evaluation of vitamin D therapy in anticonvulsant osteomalacia

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Table IV *Correlation coefficients for the seven variables used in the logistic function*

	Breathlessness at onset	SGOT quartile	Left heart failure	Relative heart size	Atrial fibrillation	Hypertension	AV block
Breathlessness at onset	1 00	0 09	0 26	0 07	0 07	−0 04	0 07
SGOT quartile		1 00	0 37	0 26	0 13	−0 07	0 [illegible]
Left heart failure			1 00	0 21	0 16	−0 00	0 25
Relative heart size				1 00	0 02	0 12	0 06
Atrial fibrillation					1 00	−0 06	0 01
Hypertension						1 00	−0 06
AV block							1 00

Table V *Coefficients for the seven variables used in the logistic function S D and ratios of coefficients and S D*

	b	s_b	b/s_b
Breathlessness at onset	0 623	0 222	2 81
SGOT quartile	0 608	0 283	2 14
Left heart failure	0 558	0 221	2 52
Relative heart size	0 547	0 206	2 66
Atrial fibrillation	0 425	0 162	2 62
Hypertension	0 345	0 214	1 61
AV block	0 207	0 167	1 24
Constant	−3 186		

a different patient series the 209 male patients with primary infarctions below 67 years of age who suffered infarction in 1971 and were cared for at the Post Myocardial Infarction Clinic were used Of these 17 died from cardiovascular causes during the 2 year follow-up Four patients died after a non fatal reinfarction during the remainder of the follow up The distribution of estimated probabilities of death was calculated The number of expected and observed deaths in each quintile was determined As information was lacking for certain variables the function was finally tested in a group comprising 195 patients of whom 17 died during two years follow-up The four patients dying after a non fatal recurrence were not excluded since this would be impracticable in future predictive applications

RESULTS

Scales and the percentage distribution for qualitative varibles in men with primary infarction are presented in Table II Table III presents the independent variables in the group of patients who died from cardiovascular causes survivors and all patients

The correlation coefficients for the independent variables are presented in Table IV The highest value (r=0 37) was found for the correlation between left ventricular failure and SGOT quartile

The ratings for the standardized coefficients (b) in the logistic model are presented in Table V together with the standard deviation (s_{b_i}) The ratio b_i/S_{b_i} can approximately be regarded as a t value for the rating The b_i coefficients presented give a rough impression of the relative role of each variable for increasing the risk of cardiovascular death Breathlessness at onset SGOT quartile left ventricular failure while in hospital relative heart size and atrial fibrillation had logistic coefficients which were significantly different from zero at the 1% level

Fig 1 presents the distribution of estimated risk of cardiovascular death in the group of male p

Table VI *Number of expected and observed deaths by decile of estimated secondary risk*

Parameter estimates according to Table V

	Decile									
	1	2	3	4	5	6	7	8	9	10
N	29	29	29	29	29	29	29	29	30	30
Estimated probability	0 004	0 007	0 012	0 016	0 025	0 035	0 063	0 116	0 233	0 457
No of deaths										
Observed	0	0	1	0	2	1	2	4	2	18
Expected	0 1	0 2	0 3	0 3	0 7	1 0	1 8	3 4	7 0	13 7

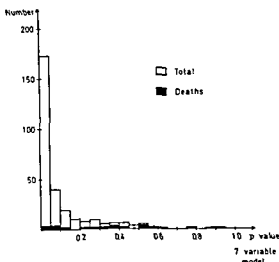


Fig 1 Distribution of patients according to estimated secondary risk during 2 years after first myocardial infarction

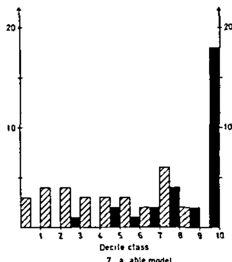


Fig 2 Number of observed cardiovascular deaths (■) and non fatal reinfarctions (▨) by decile class among patients with first myocardial infarction

tients based on 7 variables in a multiple logistic model Table VI presents the corresponding number of observed and expected deaths by decile and the expected percentage mortality It will be seen that the risk increases from 0 004 in the lowest to 0 457 in the highest decile Two thirds of deaths *occurred in the two highest decile classes and the* calculated number shows good agreement with the observed number of deaths in the deciles The distribution of observed cardiovascular deaths by decile class is presented in Fig 2 When the function was applied to the independent series of patients from 1971 the prognostic ability was found to be good (Table VII) In the two lowest quintiles two deaths occurred after a non fatal reinfarction

The estimated secondary risk of death from non cardiovascular causes is presented in Table VIII All five patients fell into the 7 lowest decile classes four below the median The estimated secondary risk for patients dying after an initially non fatal reinfarction is presented in Table IX The median risk was somewhat higher than for those who died from non-cardiovascular causes Three patients had an estimated risk above the median value

The distribution of patients with non fatal reinfarctions by decile class for estimated secondary risk is presented in Fig 2 The frequency of these recurrences was not related to the secondary risk factors for death

DISCUSSION

The importance of establishing a prognosis for death after acute myocardial infarction on the basis of variables registered during the stay in hospital has previously been stressed (28) The value of a multivariate analytical technique has also been

Table VII *Prediction of cardiovascular deaths after hospital discharge among men having sustained a first myocardial infarction*

	Quintile					
	1	2	3	4	5	Total
N	39	39	39	39	39	195
Estimated probability	0 007	0 016	0 032	0 064	0 247	
Expected deaths	0 3	0 6	1 2	2.5	9 6	
Observed deaths	1	2	0	4	10	17

Table VIII *Estimated secondary risk for non cardiovascular deaths during two years after a first myocardial infarction among men dying from non cardiovascular causes*

Cause of death	Estimated secondary risk	Decile class
Suicide	0 02	4
Suicide	0 02	4
Septicemia	0 01	2
Suicide	0 00	1
Gastric carcinoma	0 08	7

emphasized in recent years and has been used extensively (3 4 12 14, 15 18 23) Such an analytical technique makes it possible to study the prognostic significance of several variables simultaneously some of which may be interrelated which need not always be obvious at the start Many studies published so far have usually used linear methods Although a linear method can be criticized the results have demonstrated relatively good agreement with observed data For estimation of primary risk the value of logistic model rather than a linear one has been demonstrated by Wilhelmsen et al (29) and Halperin et al (10) among other authors The logistic model is also more natural for estimation of secondary risk This logistic model gives an estimation of the risk for an individual which may be said to be an expression of the probability of death for the person in question

The patients studied came from a population which was defined with respect to demographic and geographic factors and they have also been characterized in relation to the total population of the same age (1 5 6) They have been shown to constitute at least 90% of the total number of surviving myocardial infarction patients in the total population in Goteborg (7) The patients were systematically cared for after discharge by physicians who had trained together and used standardized forms of treatment (8) This reduced the variation in assessment of criteria and also permitted a more objective evaluation of the results

One fourth of the patients participated in a trial of supervised physical training (21) and 10% were treated in a controlled trial with alprenolol (30) Only about one third of the men with first infarctions in the present study participated in the experimental groups of either of the above mentioned trials In the latter study a certain influence on the mortality was reported In the present study, not more than two deaths can have been influenced by an effect of physical training or chronic β blockade This is due to the fact that only 30 men took part in the study of β blockade which demonstrated a reduction in mortality

It is unreasonable to assume that death from non cardiovascular causes for example gastrointestinal cancer and suicide is primarily associated with variables registered at the time of the infarction Deaths from non cardiovascular causes fell into low decile classes The analysis was therefore limited to cardiovascular deaths The precision of prediction was thereby increased Only a few deaths during two years follow up after infarction were due to non cardiovascular causes (2 24 27) Restriction to pure cardiac deaths instead of all cardiovascular deaths would not have conferred any advantage This is due to the fact that cardiac deaths dominate the cardiovascular group and that cardiovascular non cardiac deaths for example cerebrovascular lesions often constitute the immediately fatal episode after a complicated infarction in other cases (24) At the first calculation of the predictive model patients who died after an initially non fatal reinfarction were excluded Previous results have shown that these patients had a higher 2 year mortality than other patients in the series (23) It could therefore be assumed that the infarction at entry to the study was only partially decisive of death However the estimated risks for this group fell to a considerable degree into the upper risk classes This reduces the predictive error when the risk function is applied prospectively when patients with non fatal reinfarctions cannot be

Table IX *Estimated secondary risk for patients dying after an initially non fatal reinfarction and the intervals initial infarction–reinfarction and infarction–death*

Pat no	Estimated secondary risk	Decile class	Interval from initial infarction (mo) To reinfarction	To death
1	0 58	10	1	10
2	0 80	10	21	23
3	0 03	5	11	13
4	0 02	4	4	5
5	0 09	8	16	21

xcluded in advance Thus the predictive precision if a prognostic function using variables from the initial hospital phase is always reduced by the occurrence of non fatal reinfarctions Therefore the period of study has to be limited

The problem in the present study was to find suitable predictors After a first manual selection further reduction was achieved by linear analysis and a logistic model was then used for the prediction If the effect of interactions other than those taken into consideration in the model had been included in the calculations problems would rapidly have arisen The number of parameters would have been very large and the only possibility was to reduce the number of variables

Age was not found to differ essentially between patients who died and those who survived in the age range studied (Table I) The upper age limit also reduced the occurrence of age related interactions Elderly individuals are more likely to have several diseases concurrently and the pattern of causes of death is more complicated

All secondary risk factors found to have low *p* values might be associated with the extent of myocardial necrosis (Table II) The variables studied showed high correlation coefficients in several cases (Table IV) Despite this the factors made independent contributions to the predictive capacity Many factors which distinguished patients who died and those who survived (Table I) (25) may be associated with those in the logistic model which were interesting here

The factors which were included in the final analyses in the present investigation partly accorded with those shown to be secondary risk factors in other multivariate analyses (4 12 14 18) Direct comparisons between studies are difficult Patients included in other studies are not comparable with respect to age distribution sex previous infarctions duration of follow up etc In addition the patients in the present study were systematically cared for during the subsequent course

Analyses aimed at establishing secondary risk factors in different series can however give widely differing results (4) despite similarities with respect to patient selection the composition of the patient series and follow up conditions An important factor is that the variables are strongly interrelated and that our knowledge of the relationships is incomplete (9) The causes of death after myocardial infarction are complex and vary with the duration of follow up The acute infarction and its complications are of importance and factors which caused the first infarction and which continue to exert an influence are also involved

The majority of deaths in this study were sudden i e they occurred within 24 hours (24) They could be predicted without utilizing arrhythmia variables from tape recorder registration before discharge which was not available but has been found to provide useful information in other studies (14)

Assessment of the efficiency of the predictive function is only possible if it can be tested in another similar patient series in which a number of deaths occur during an equally long follow up (Table VII) The good prognostic ability demonstrated for the risk function may partly be due to the analysis being limited to men with first infarctions aged below 67 years who died from cardiovascular causes during a limited period of follow up (two years)

Patients with previous myocardial infarction have a pronounced excess mortality from reinfarction in relation to the normal population The mortality is 10–30 times as high during the first two years after infarction (24 26) Similarly there is an excess morbidity in initially non fatal reinfarctions amounting to the same magnitude (24) First infarctions in the population of Göteborg have previously been shown to be predicted by a logistic function that includes the variables systolic BP smoking and serum cholesterol (29) The distribution of the so-called primary risk estimated in this way is the same for all patients with first infarctions for those who die after the infarction and for patients with non fatal reinfarctions (23)

Irrespective of which factors are causal in myocardial infarction it is reasonable to regard surviving patients as manifestly sensitive to these factors This may be utilized in order to make trials in secondary prevention more effective Patients with high mortality risk can be utilized for secondary preventive trials with death as the main end point Individuals with low mortality risk can be used for studying possible causal relationships for example modification of primary risk factors with non fatal reinfarction as the main end point

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Family Study of High Density Lipoprotein Cholesterol and the Relation to Age and Sex

The Tromsø Heart Study

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ABSTRACT A family study of serum high density lipoprotein (HDL) cholesterol and total serum cholesterol concentration has been undertaken and the relation to age, sex cigarette smoking physical activity and familial occurrence of myocardial infarction (MI) was examined HDL cholesterol was determined in 251 females and 194 males and total serum cholesterol in 677 females and 657 males all aged 0–49 years With respect to HDL cholesterol, significant sex differences were observed both in absolute level and in age related change A negative correlation between HDL cholesterol and total serum cholesterol was observed in all age groups except females aged 0–19 years supporting the hypothesis of HDL as a "clearing ' lipoprotein HDL cholesterol showed a positive correlation only in pairs of first degree relatives involving the mother and in sib-sib pairs of the same sex On the other hand for serum cholesterol a positive correlation was found among all family members although significantly higher between first-degree relatives than between spouses No relation was found between cigarette smoking physical activity or familial occurrence of MI and the HDL cholesterol or total serum cholesterol concentrations In accordance with the HDL hypothesis the present finding could partly explain the higher incidence of ischaemic heart disease (IHD) in males than in females and partly also the high risk which is transmitted from women with IHD to their first-degree relatives

Epidemiological surveys have demonstrated positive correlations between the future incidence of ischaemic heart disease (IHD) and plasma concentrations of total cholesterol (7 17) and triglyceride (7) The major cause of IHD coronary atherosclerosis is characterized histologically by the accumulation of lipid predominantly cholesterol in the arterial wall together with a local connective tissue reaction (1) Cholesterol is transported in plasma predominantly as a component of low density lipoprotein (LDL) but also in the triglyceride rich very low density lipoprotein (VLDL) and in the high density lipoprotein (HDL) fraction There is experimental evidence that the relationship of atherosclerosis to hypercholesterolaemia and triglyceridaemia may be due to the infiltration into the arterial wall of LDL and VLDL respectively with resultant deposition of cholesterol (18 33)

The plasma concentration of HDL on the other hand appears to be inversely related to coronary risk (14 22) suggesting that HDL may retard atherogenesis possibly by facilitating cholesterol transport from the vessel wall (4) or by inhibiting the cellular uptake of LDL (19)

Although there is considerable information in the literature concerning plasma concentrations of cholesterol triglyceride and LDL in different populations and their relationships to environmental and genetic factors little such data are published with regard to HDL In the present study the influence of age sex physical activity smoking habits and positive family history of myocardial infarction (MI) on the total plasma cholesterol and HDL cholesterol concentrations has been examined in a population sample living in Northern Norway Familial correlation analysis of the same two variables was also performed

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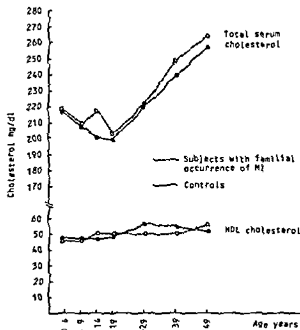


Fig 1 Mean total serum cholesterol and HDL cholesterol in females with familial occurrence of myocardial infarction and controls

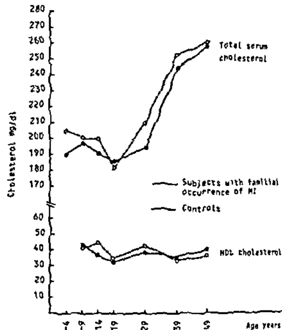


Fig 2 Mean total serum cholesterol and HDL cholesterol in males with familial occurrence of myocardial infarction and controls

MATERIAL AND METHODS

In a population survey (32) for coronary risk factors in the City of Tromsø Northern Norway 129 men who reported MI both among their own and their wives first-degree relatives brought their spouses and children for serum cholesterol determination In addition to the couples 122 daughters and 125 sons were examined Despite the fact that the positive family histories at this stage were not verified these families were considered as the group with familial occurrence of MI In this group subjects aged 0–19 years had MI among their grandparents on both sides of the family whereas those above this age had MI among first-degree relatives

As a control group 400 married men aged 35–49 years were randomly selected from the survey population and asked to bring their spouses and children for similar examination Of these 316 married couples with 361 daughters and 341 sons participated The blood samples were collected throughout the year There was no systematic accumulation of subjects from the two groups at any time of the year No instructions were given concerning dietary habits before the examination Subjects with recent acute illnesses were asked to report for examination two weeks after recovery No clinical examination was performed and no history of past illnesses was taken at the examination

The measurements of total cholesterol concentration in serum were performed according to a Liebermann Burchard procedure using the reagent described by Huang et al (16) Details of the procedure and quality control have been published elsewhere (32) The determination of HDL cholesterol was introduced somewhat later in the study and was performed in 139 females and 110 males among the subjects with familial occurrence of MI and in 112 females and 84 males in the control group Serum HDL cholesterol concentration was measured in the supernatant after precipitation of VLDL and LDL according to Burstein et al (5) S E M of consecutive duplicate analysis in 10 samples was 3% The significance of differences between the regression coefficients was calculated according to the formula

$$t=\frac{b_1-b_2}{\sqrt{S\,D_1^2+S\,D_2^2}}$$

where b_1 and b_2 are the regression coefficients and S D $_1$ and S D $_2$ the respective standard error of b

Classification of subjects into groups with different smoking habits and physical activity was done on the basis of information collected in the primary survey (32) Subjects reporting daily cigarette smoking were classified as daily cigarette smokers whatever their daily consumption The mean daily cigarette consumption was 14 7 cigarettes (32) Subjects who reported taking regular physical exercise more than four hours a week in their leisure time were classified as having high physical activity the other as belonging to the sedentary life group In tables where no age groups are given the values have been age-adjusted according to the indirect method (2)

The correlation coefficients between relatives were estimated on the basis of an adjusted value for each person according to the formula $(x_i-x)/s$ where x_i is the subject s observed value x the estimated sex specific mean value for the subject s ten year group and s the standard deviation of this mean This calculated value denotes the sub-

Table I *Mean total serum cholesterol and HDL cholesterol according to age and sex*

Age (y)	Males			Females		
	N	mg/dl	S D	*N*	mg/dl	S D
Total cholesterol						
0– 9	171	203 0	33 8	176	209 4	34 1
10–19	280	192 2	33 0	278	199 0	38 5
20–29	38	210 6	44 0	70	223 0	55 7
30–39	144	264 7	49 0	210	249 7	47 9
40–49	288	279 7	53 4	198	271 1	54 0
HDL cholesterol						
0– 9	36	52 6	13 2[a]	37	46 4	11 1
10–19	61	47 0	10 3	76	49 5	9 4
20–29	11	52 2	14 2	24	50 6	9 8
30–39	33	44 2	12 0[b]	58	51 6	12 1
40–49	53	47 9	11 7	56	52 4	13 3

[a] $p<0\ 05$ compared with females of the same age
[b] $p<0\ 01$ compared with females of the same age

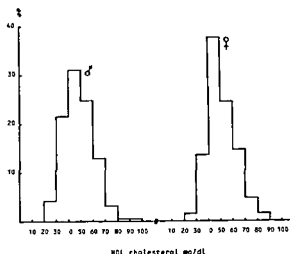


Fig 3 Frequency distribution of HDL cholesterol in males and females

ject s relative position within his own sex and age specific distribution measured in standard deviation units Confidence limits for correlation coefficients are taken from Geigy Scientific tables (29) Statistical significance of differences between means has been estimated by using a large sample *t* test *P* values higher than or equal to 0 05 were regarded as not significant

RESULTS

Only minor differences were observed in total serum cholesterol and HDL cholesterol concentration between subjects with familial occurrence of MI and controls (females Fig 1 males Fig 2) Using regression analysis of the variables upon age and comparing the means within five year age groups the only significant difference observed was an elevated total serum cholesterol concentration in females aged 10–14 years who had MI among their grandparents on both sides ($p<0\ 01$) Despite this difference the two groups of families were pooled for further analysis Fig 3 shows the frequency distribution of HDL cholesterol The distribution is approximately normal for both sexes though with a weak right skewness

Tables I and II show mean total serum cholesterol HDL cholesterol and the estimated ratio HDL cholesterol/total cholesterol – HDL cholesterol (referred to as the ratio) according to age and sex Males aged 0–9 years had higher HDL cholesterol and ratio than females at the same age ($p<0\ 05$) whereas the opposite pattern was found in age group 30–39 years ($p<0\ 01$)

Figs 4 and 5 give the change of HDL cholesterol

Table II *Estimated ratio (HDL cholesterol×100)/(total cholesterol – HDL cholesterol) by age and sex*

Age (y)	Males			Females		
	N	Ratio	S D	*N*	Ratio	S D
0– 9	36	36 1	11 2	37	30 2	8 7
10–19	61	32 3	12 4	76	33 7	10 9
20–29	11	32 7	14 7	24	31 9	10 4
30–39	33	21 7[b]	7 1	58	28 1	11 8
40–49	53	23 4	10 8	56	25 9	10 5

[a] $p<0\ 05$ compared with females of the same age
[b] $p<0\ 01$ compared with females of the same age

Table III *Mean HDL and mean ratio (HDL cholesterol×100)/(total cholesterol – HDL cholesterol) in men aged 20–40 years according to smoking habits and physical activity (age-adjusted values)*

	N	HDL (mg/dl)	S D	Ratio	S D
Sedentary life	59	47 3	13 2	23 8	11 8
High physical activity	32	46 6	11 7	23 8	8.3
Daily smokers	53	47 0	12 5	23 6	10 7
Non smokers	37	47 6	13 1	24.0	11 0

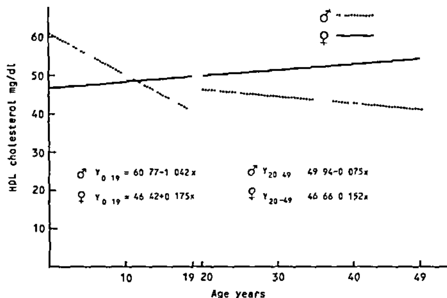


Fig 4 Regression lines of HDL cholesterol upon age in males and females

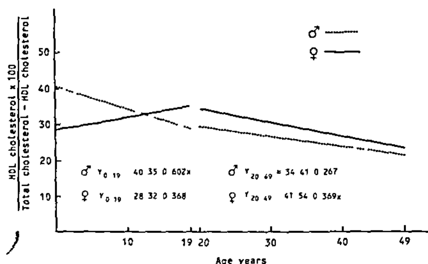


Fig 5 Regression lines of the ratio

$$\frac{\text{HDL cholesterol} \times 100}{\text{total cholesterol} - \text{HDL cholesterol}}$$

upon age in males and females

and the ratio with age for both sexes The regression is calculated separately for the age groups 0–19 and 20–49 Males aged 0–19 years had a significant decline both in HDL cholesterol ($p<0\ 01$) and in ratio ($p<0\ 05$) Neither HDL cholesterol nor the ratio changed significantly with increasing age in males aged 20–49 years In females aged 0–19 and 20–49 years the HDL cholesterol rose slightly but not significantly with age The ratio increased in females aged 0–19 years whereas it fell significantly ($p<0\ 01$) during the following three decades Table III shows that neither physical activity nor smoking habits had any influence on the HDL cholesterol or the HDL/total cholesterol – HDL cholesterol ratio

Table IV shows a negative correlation between HDL cholesterol and total cholesterol – HDL cholesterol in males aged 0–19 ($p<0\ 05$) and 20–49 years ($0\ 05<p<0\ 1$) and females aged 20–49 years ($p<0\ 01$) In females aged 0–19 years the correlation coefficient is significantly different ($p<0\ 01$) from that in the other groups

Serum concentration of HDL cholesterol showed a positive correlation only in pairs of first degree relatives involving the mother and in sib-sib pairs of the same sex where a remarkably high correlation was found (Table V) On the other hand total serum cholesterol showed a significant correlation between all pairs of first degree relatives and spouses The coefficients however were significantly higher between all first-degree relatives than between spouses ($p<0\ 05$)

DISCUSSION

The negative correlation found here between serum concentration of HDL cholesterol and total cholesterol–HDL cholesterol supports the hypothesis put forward by Miller and Miller (22) that HDL is a clearing lipoprotein for cholesterol from the arterial wall

The positive correlation observed in pairs of family members involving the mother and in sib-sib pairs of the same sex suggests that the intrafamilial resemblance in HDL cholesterol is sex associated This pattern is not consistent either with sex linked or with single gene dominant or recessive inheritance Since the correlation coefficients are influenced by both environmental and genetical factors any categorical interpretation of these results would be hazardous However the mother linked association may help to explain the high risk for IHD that is transmitted from females with IHD to their first degree relatives (30) The familial study of hyper-α lipoproteinemia by Glueck et al (14) showed correlation coefficients of HDL cholesterol between sib sib pairs of the same order of magnitude as those observed by us Differences in the selection of subjects within the two studies however makes it difficult to compare offspring parent correlation coefficients

The higher correlation between first-degree relatives than spouses supports the theory of a genetic influence on the total cholesterol concentration No sex associated genetic influence was found for total cholesterol The correlation observed among first degree relatives is in accordance with the findings of Schaefer et al (28) and Godfrey et al (15)

In agreement with earlier studies (10 24) the present results confirm that after adolescence females have a higher HDL cholesterol level and a higher HDL/total cholesterol–HDL cholesterol ratio than males The sex difference starts at puberty reaches its maximum at the age of 30–39 years and gradually diminishes at 40–49 years A higher ratio among women after adolescence may help to protect them for a longer period of life against the deleterious effect of environmentally induced high LDL cholesterol It is noteworthy that the serum concentration of HDL cholesterol and ratio were specially low in males aged 30–39 years compared with females in the same age group This may indicate a period of life in males with a relatively inefficient clearance mechanism for cholesterol As the sex difference starts at puberty it may possibly be linked to hormonal changes e g increased estrogen level in females and/or increased androgen activity in males (27) Regrettably we do not know anything about the use of contraceptive pills in the population studied

The higher HDL cholesterol and ratio in males before puberty than in females of the same age is a new observation that has not been reported in earlier publications (3 11 31) Neither the interpretation nor the importance of this finding are clear

Several conditions with increased incidence of IHD have been associated with reduced plasma concentration of HDL cholesterol (6 9 13 22 24 34) Although many of these conditions are genetically influenced no association was observed between the familial occurrence of IHD and HDL cholesterol

The lack of a difference in HDL cholesterol between subjects with sedentary physical habits and those with high physical activity in leisure time is in contrast to earlier studies showing higher HDL cholesterol level after physical training (21 35)

Table IV *Correlation coefficient between HDL cholesterol and total cholesterol – HDL cholesterol according to age and sex*

Age (y)	Males N	Males r	Females N	Females r
0–19	96	−0 20	113	0 15
20–49	97	−0 18	138	−0 25

Table V *Correlation coefficients for total serum cholesterol and HDL cholesterol between first degree relatives and spouses*

	Total cholesterol		HDL cholesterol	
	No of pairs	r	No of pairs	r
Mother–father	412	0 13	91	0 15
Mother–son	500	0 34*	109	0 36*
Mother–daughter	511	0 24*	126	0 32*
Father–son	508	0 27*	82	0 21
Father–daughter	518	0 24*	87	0 01
Son–daughter	211	0 44*	36	0 18
Son–son	236	0 43*	40	0 51*
Daughter–daughter	220	0 30*	43	0 56

Possibly the questionnaire used in the present investigation failed to discriminate between subjects with high and low physical activity

A reduction of plasma HDL levels has been reported in males smoking 22 5 cigarettes a day compared with non smokers (26) The fact that our study did not demonstrate any difference in HDL cholesterol between smokers and non smokers may be due to markedly lower daily cigarette consumption by the smokers in our material than in the above study

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The Prognosis in Ebstein's Disease of the Heart

Long term Follow up of 22 Patients

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ABSTRACT A follow up study of 22 patients with Ebstein s anomaly has been performed Nine patients died 1–21 years (mean 9) after the initial admission while the 13 patients alive at the end of the observation period had been followed for 5–26 years (mean 15) Clinical ECG, radiological, and haemodynamic features were analyzed with reference to their prognostic significance The presence or absence of cyanosis due to right to left shunt through an atrial septal defect (ASD) distinguished best between a good and a poor prognosis Right sided heart failure and dyspnoea at rest, often associated with palpitations precordial pains and syncopes were grave prognostic findings After the initial signs of heart failure there was a rapid deterioration death ensuing within a few years Operation with insertion of a prosthetic valve (and closure of the ASD) should be seriously considered at the appearance of heart failure

An increasing interest has been directed towards the natural history of Ebstein s anomaly (1 6 8) due to several reports about successful correction with insertion of a prosthesis in the tricuspid ostium

As Ebstein s anomaly is rare few cases are seen in each department—of 61 centres in the co-operative study guided by Watson (8) only 17 collected more than 10 cases—and the observation time is often short We have followed 22 patients with Ebstein s anomaly for 5–26 years

The purpose of this paper is to describe factors of importance for the prognosis based on these 22 patients and the literature The diagnostic problems have been presented in other papers from our department (4 5) and in several extensive reviews (1 3 6 7 8) and will not be dealt with here

MATERIAL AND METHODS

In the years 1948–68 23 patients with Ebstein s anomaly were admitted to Rigshospitalet and Queen Louise s Childrens Hospital Copenhagen Clinical ECG and radiological features on admission in 20 of them have been reported previously (5) The diagnosis was in all cases based on haemodynamic and angiocardiographic examinations All patients alive were readmitted during 1971–75 to Rigshospitalet A last follow-up was carried out with a questionnaire through public registration offices Death certificates were procured for all patients who died in the observation period One patient was British and was lost for follow-up Our study is thus based on 22 patients On first admission and at check ups in the hospital 12 lead ECGs were recorded and biplane chest X rays taken Six patients have been in the department only once additional information about them was obtained from local hospital records Only chest X rays from our Department of Radiology were used in the follow up

The observation time for 13 patients alive at the end of the period (summer 1975) was 5–26 years (mean 15) and for nine patients who died in the period 1–21 years (mean 9)

The patients were divided into two groups (Table I) Group A 12 patients with Ebstein s anomaly and atrial septal defect (ASD) Six were male and six female Median age on the first admission was 10 years (range 1–36) Group B 10 patients with Ebstein s anomaly without ASD Eight were male and two female Median age on first admission was 24 years (range 5–55)

RESULTS

Clinical features

Group A Table I shows that nine of 12 patients had cyanosis and/or dyspnoea on exertion from birth All patients had dyspnoea on exertion at age seven and all but one had cyanosis at age 12 the exception developing cyanosis at age 37 Palpitations precordial pains and syncopes are seen in a later

Table I *Clinical features of 22 patients with Ebstein s anomaly*
HF=heart failure VT=ventricular tachycardia S=sudden death PO=postoperative

	Group A											
Status on admission												
Age (y)	1	3	3	7	8	10	10	12	18	22	28	36
NYHA functional class	II	II	II	II	II	II	III	II	II	II	II	II
Status at follow up												
Age when alive (y)	10[a]	–	26[a]	–	–	–	15	–	–	43	–	42
NYHA functional class	I[b]	–	II[b]	–	–	–	III	–[b]	–	II	–	III
Age at death (y)	–	6	–	24	14	19	–	33[a]	26	–	38	–
Cause of death	–	HF	–	HF	S	HF	–	PO	VT	–	HF	–
Age (y) at start of												
Cyanosis	0	0	0	0	0	10	0	12	0	0	0	37
Exertional dyspnoea	0	0	0	0	7	6	0	0	0	0	0	7
Dyspnoea at rest	7	4	22	23	13	18	–	32	–	–	37	42
Palpitations	–	–	22	23	13	13	–	–	20	20	36	–
Precordial pains	–	–	–	–	13	13	–	6	–	–	36	36
Peripheral oedema	7	4	22	23	–	18	–	32	–	–	37	42
Syncopes	–	5	–	23	12	–	–	32	–	–	36	–
Cerebral emboli	–	–	–	14&20	–	–	–	–	–	–	–	31

[a] Operation [b] In functional class IV before operation

period of life (at ages 5–36) Peripheral oedema dyspnoea at rest and increasing cyanosis are very grave prognostic signs seen from the fourth to the 42nd year of life These signs were followed by death within 24 months in six of the seven patients who died in this group and were present before operation in the two patients who were treated ccessfully with insertion of a tricuspid valve sthesis Two patients had cerebral emboli in ne followed by severe haemiparesis Two severely cyanotic patients had periods with haemoptysis

Seven patients died four from heart failure one from an attack of ventricular tachycardia lasting for 12 hours one patient who had previously had several syncopes was found dead one died post operatively

Five patients were alive at the end of the ob servation period One has been followed for 21 years with unchanged functional status the patient is now 43 years old in NYHA functional class II Two patients now 15 and 42 years of age have developed increasing symptoms and are now in NYHA class III Two patients aged 10 and 26 both in functional class IV before operation are in classes I and II following operation

Three patients were operated on In one a Bla lock operation was performed at age five followed by a considerable improvement This patient was readmitted 22 years old in NYHA functional class IV She was operated again with insertion of a pros thesis in the tricuspid ostium and closure of the ASD Four years after the operation she is now acyanotic and incapacitated only by slight dyspnoea on exertion The second patient had slightly in creasing symptoms until at the age of seven he developed right sided heart failure several syn copes (cyanotic spells) and severe haemoptysis and was bedridden The patient was operated on when eight years old and is now two years later without complaints The third patient was followed from his 12th year At the age of 32 he was in functional class IV like the other two patients The operation was initially successful but had to be repeated four months later because of tricus pid insufficiency and the patient died postopera tively

Group B None of the patients had symptoms at birth The first complaint i e dyspnoea on exer tion appeared in the 2nd and 3rd decade and was followed about 10 years later by palpitations and precordial pains in conjunction with exercise Two patients had cerebral emboli with slight paresis followed by nearly complete recovery

Two patients died one from right sided heart failure 61 years old and the other was found dead in his home 44 years old

Eight of ten patients were alive at the end of the observation period In seven patients no change

Group B									
5	7	10	13	22	26	26	30	43	55
I	*I*	*II*	*I*	*II*	*II*	*II*	*II*	*II*	*II*
31	13	31	21	44	45	40	41	–	–
III	I	II	I	II	II	II	II	–	–
–	–	–	–	–	–	–	–	44	61
–	–	–	–	–	–	–	–	S	HF
–	–	–	–	–	–	–	–	–	–
29	–	10	–	21	17	13	30	20	54
–	–	–	–	–	–	–	–	–	57
1	–	20	–	21	26	–	31	43	35
–	–	20	–	21	26	–	39	39	–
–	–	–	–	–	–	–	–	–	57
–	–	–	–	–	–	–	–	–	–
–	–	17	–	–	–	32	–	–	–

was seen in the functional capacity during a period of 6–22 years One patient with WPW block has had attacks of supraventricular tachycardia from his first year of life in the last four years with increasing frequency and duration often provoked by exercise He is now 31 years old ı NYHA functional class III

Electrocardiogram

Group A All patients had sinus rhythm on the first admission ECGs were recorded at intervals of 3–20 years in 11 patients The QRS complexes were unchanged in all but one patient who had WPW block *in 1953 and RBBB in 1971 All* patients had sinus rhythm at the follow up Supraventricular tachycardia has been recorded in five patients four of these patients complained of palpitations and one had WPW block One patient died after an attack of ventricular tachycardia lasting for 12 hours

Group B One patient 55 years of age had atrial fibrillation all the time from first admission until death six years later Judging from information from the local hospital it is most likely that he had had atrial fibrillation from his 35th year One year after the first admission at age 27 another patient developed atrial fibrillation which still persisted 19 years later

The other eight patients had sinus rhythm after a follow up period of 6–26 years One patient had an attack of atrial fibrillation at right heart catheterization when 42 years old and stated that he had had the same type of attacks for the last ten years One patient with WPW block had intermittent supraventricular tachycardia with increasing frequency and duration often provoked by slight exercise The QRS complexes were unchanged in all patients

Chest X ray

All but two of the patients had enlarged cardiac silhouettes on the chest X ray at the first examination Heart volume varied between 360 and 1920 ml/m² BSA average 730 (S D 350) (normal upper limit 450) In patients with the slightest enlargement there was usually just a prominence of the lower part of the left heart border in the frontal view In the lateral view there was invariably a prominence of the upper anterior border of the heart shadow In patients with moderately enlarged hearts the typical finding was a box like appearance of the heart in the a p projection with a narrow vascular pedicle and a small aortic arch segment (2) The largest heart shadows were almost spherical in both projections In five patients (3 with ASD) the lung vessels were of normal size In the remaining 17 patients the central and peripheral vessels appeared small

The average heart size was 700 ml/m² BSA (S D 299) in patients with ASD and 760 (S D 410) in patients without ASD a statistically insignificant difference The nine patients who died had an average heart size of 990 ml (S D 458) against 574 (S D 119) for the remaining patients at the first examination ($p<0\ 05$)

In seven patients no follow up (i e chest X rays taken at an interval of at least one year) was available in six patients due to early death In the remaining 15 patients at least two but often several chest X rays were available at intervals of 2–26 years (average 5 6)

In five patients heart size was unchanged (change less than 10%) during the follow up period of 2–19 years In the others an increase in size of 10–120% (average 41) was observed With a few exceptions the greatest increase in heart size was observed in the patients who were followed for the longest period As regards increase in heart size patients with ASD did not differ from those without ASD

Haemodynamic findings

A right heart catheterization was performed in all patients In one patient the right ventricular systolic pressure was 56 mmHg because of pulmonary stenosis All other patients had normal right sided pressures At the first examination the oxygen saturation in the pulmonary artery varied from 55 to 76% in patients with ASD and from 58 to 78% in patients without ASD No difference was found between patients who died and who survived the observation period

A second right heart catheterization was performed in 10 patients 5–20 years after the initial one Neither in five patients with ASD nor in five without ASD were significant changes seen in the pressures or in the pulmonary or systemic artery oxygen saturation

DISCUSSION

It is evident from the literature that Ebstein s anomaly has a broad clinical spectrum Some patients live a completely normal life the anomaly being an incidental finding at autopsy The oldest patient reported was 79 years old (7) Some patients have severe symptoms from birth and die after a few months or years (8) Of 219 patients reported in the literature and collected by Makous and Vander Veer (7) 50% died before the age of 7 Of 43 patients first seen between birth and five years of age Kumar et al (6) found that 50% survived to 13 years of age

The difference in survival between the patients reported by Kumar et al (6) and by Watson (8) is explained by the larger number of patients below the age of two when first admitted among those reported by Kumar et al Watson found a one year mortality of 62% in patients admitted in the first year of life and Kumar et al a mortality of 33% within the first two years of life in 34 patients Only one of our patients was below two years of age on the first admission and the prognostic problems related to infancy are not illustrated in our material

It is concluded from the literature that life expectancy is considerably reduced in patients with Ebstein s anomaly as a whole Because of the great differences in survival it is important to identify the factors which determine the prognosis and in this way describe the patients in whom operation should be considered and define the optimal time for surgical correction

Our study demonstrates that cyanosis caused by right to left shunt through an ASD distinguishes best between patients with a good and patients with a poor prognosis Of 12 patients with ASD seven died in the observation period and only two survived because of radical operation the mean age at death being 22 years Of the 10 patients without ASD only two died at the age of 44 and 61 This finding is in accordance with Kumar et al (6) who report that the eight year survival rate after the first admission was 94 60 and 21% in patients without with moderate and with severe cyanosis respectively

In accordance with others (1 6 8) we found a slight correlation between increasing heart size on chest X ray and mortality But as several patients with markedly enlarged hearts are still alive 20 years after the first admission heart size is not a good determinant of the prognosis

Neither the ECG nor the haemodynamic investigations were of independent value in establishing the prognosis Kumar et al (6) suggest that a high amplitude of P_{II} presages limited survival We agree with this The explanation is that patients with ASD have the highest amplitude of P_{II} In our patients we found $P_{II}>0\ 25$ in nine of 12 patients with ASD and $P_{II}\leq 0\ 25$ mV in eight of nine patients without ASD, excluding the one with atrial fibrillation The tall P wave is caused by right atrial hypertrophy and the degree of hypertrophy is dependent on the severity of the functional tricuspid stenosis the worse the stenosis the greater the atrial hypertrophy and the right to left shunt and the worse the prognosis

Signs of right sided heart failure dyspnoea at rest often associated with palpitations syncopes and precordial pains are very grave prognostic findings These symptoms and signs appeared in our patients between the ages of four and 57 Irrespective of the age at appearance none of the patients survived four years and none of the cyanotic patients survived two years after the initial signs of heart failure (excluding the two patients who survived operation) When heart failure appears a rapid deterioration should be expected (6)

According to the literature (6 8) 20% of the deaths are sudden Among our patients two of nine died suddenly One of the patients with ASD had several syncopes prior to death The other patient complained of palpitations and this together with one death caused by ventricular tachycardia sup-

ports the view that sudden death is caused by tachy arrhythmic disturbances (6 8)

It has not been possible to establish ECG changes which might help to find patients susceptible to sudden death But it is reasonable to suggest that WPW block seen in 5–10% of the patients may be one factor of importance

Finally it should be remembered that systemic emboli represent a severe risk in these patients although it is not possible to give exact figures on the degree to which this affects the prognosis

Watson (8) stresses that operation with insertion of a prosthesis in the tricuspid ostium still carries a great risk the lethality was about 50% in the un complicated cases As demonstrated by the results in two of the three patients operated on in our se ries successful operations are possible

Based on reports in the literature and our own experience as presented above we conclude that most of the patients with Ebstein s anomaly ad mitted for the first time after infancy have no or few complaints for many years Patients with cyanosis have the shortest life expectancy A rapid deteriora tion leading to death within one to two years is found after appearance of heart failure Operation with insertion of a prosthesis in the tricuspid ostium and closure of the ASD should then be seriously considered

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Low Threshold Endocardial Electrodes for Permanent Cardiac Pacing

Comparison between One Large and Two Small Surface Electrodes

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ABSTRACT Three different electrodes i e conventional large and small surface endocardial electrodes and a new large contact area–small active surface electrode have been compared with respect to their stimulation thresholds for cardiac pacing in 94 patients with 96 electrodes Both capacitor discharge and constant current pulses were used for the measurements The average 2 msec impulse threshold for the 47 mm² electrode reached its maximum on the 14th postoperative day and was 3 6±1 4 V and 4 4±1 8 mA One month after insertion the threshold was 3 2±1 4 V and 3 9±1 7 mA Corresponding values after one month for the 6 mm² electrode were 2 0±1 0 V and 1 7±0 9 mA and for the new electrode 1 7±0 9 V and 1 4±0 8 mA Thresholds increased by about 20% when the impulse duration was diminished from 2 to 0 5 msec The new large area–small active surface electrode offers the advantages of significantly smaller increases in stimulation thresholds during the first month after insertion and good attachment to the endocardium

The transvenous endocardial electrode was introduced in 1959 (12) adapted to an implanted system in 1962 (17) and is now the most important electrode for cardiac pacing (29) Its main disadvantages however are relatively frequent dislocations and increases in the stimulation thresholds (5 15 30) Therefore several modifications have been suggested to improve its attachment to the myocardium (1 2 24) and to decrease the rise in stimulation threshold by increasing the current density through the reduction of electrode surface area (11 19 26) The stimulation threshold is dependent not only on the size of the electrode but also on the distance between electrode and excitable heart muscle (20 28) Due to thrombotic depositions and growth of fibrous tissue around the electrode tip this distance will increase during the first few postimplantation weeks resulting in an elevated stimulation threshold but also anchorage of the electrode to the myocardium (3 14 16 22)

The aim of the present investigation was to compare the threshold curves and other features during the first month after insertion of conventional electrodes with large and small surfaces with those of a new modified electrode with the tip in the shape of a cage providing a large contact area but a small active surface For threshold measurements the capacitor discharge pulse form (voltage pulse) is widely used in Europe whereas the constant current pulse is mostly employed in the USA Both pulse forms were utilized in this investigation to facilitate comparisons

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PATIENTS AND METHODS

The patients were provided with a permanent transvenous endocardial electrode during the period Sept 1973 – May 1975 in the Department of Surgery Serafimerlasarettet Stockholm The operations were performed by one of the authors (H L) Altogether 115 electrodes were implanted in 113 patients For the reasons given in Table I 19 patients were excluded from the study This left a study group of 94 patients with 96 electrodes

Three different types of unipolar endocardial electrodes were used (Fig 1) and the patients were grouped accordingly The electrodes were randomly distributed among the patients mainly depending on which type was readily available at the time of operation Group A consisted of 22 patients who received a Siemens Elema electrode with a 47 mm² platinum tip (EMT 588 A) Group D comprised 37

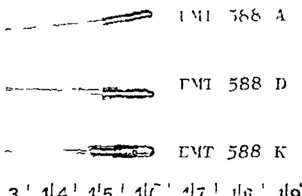


Fig 1 The three different types of electrodes EMT 588 A with a 47 mm² EMT 588 D with a 6 mm² and EMT 588 K with a 8 mm² surface area

patients with the same electrode as in group A but with a modified tip partly insulated with a thin silicon rubber membrane to decrease its electrically active surface to 6 mm² (EMT 588 D) The third group of 42 patients received a newly designed electrode with the platinum tip in the form of an empty wire cage with an electrically active surface of 8 mm² and the rest of the metal insulated with carbon pigmented epoxy resin (EMT 588 K) The purpose of the carbon pigmentation was to reveal insulation defects

There were 31 women with a mean age of 75 years (range 57–84) and 63 men mean age 74 years (range 44–93) Their data are presented in Table II In 65 of the patients permanent pacing was started on account of syncope in connection with AV block sinoatrial block tachy or bradyarrhythmias The remaining 29 patients had the same kind of conduction disturbances in combination with heart failure and/or dizziness but no syncopes (Table III) Six patients had had earlier implantations Two of them had their previous A electrodes replaced by D electrodes on account of threshold rises during the first postoperative month and four had to have their electrodes exchanged after more than 8 years because of exit blocks The other 90 electrodes were original insertions

The operations were performed applying a two stage procedure the electrode being connected at first to an external pulse generator and about one month later to a subcutaneously implanted pacemaker (18) Thresholds were measured with a special threshold analyzer equipped with two alternative output stages one giving a constant current pulse and the other a capacitor discharge pulse The output capacitor in the latter case could be adjusted between 2 2 and 33 μF in 10 stages but for this study only the 3.3 μF capacitor was used The pulse width and rate could be adjusted from 0 to 10 msec and from 0 to 200 bpm respectively The ranges for the two output stages were adjustable within the range of 0–10 mA and 0–10 V respectively All parameters had an accuracy of 2% The measurements were routinely performed at a stimulation rate of 95 bpm If however the patient had a higher spontaneous heart rate stimulation rates up to 125 bpm were used

Stimulation threshold was defined as the lowest impulse amplitude delivered with decreasing intensity capable of producing regular QRS complexes registered with ECG on oscilloscope The thresholds were measured immediately after introducing the electrode and again on the 3rd 7th 14th and 30th postoperative days in each patient at the same time of day Most of the patients were ambulatory after the third day

The significance of differences between mean values was tested by Student s *t* test Degrees of significance were tested at the 5 1 and 0 1% level

Table I *Reasons for excluding 19 patients from the study in relation to electrode type*

	Electrode			No of pats
	A	D	K	
Incomplete data	2	6	3	11
Died within 1 month (no pacemaker failure)	2	0	3	5
Transferred to another hospital	0	1	0	1
One stage procedure at implantation	0	1	1	2
Total	4	8	7	19

Table II *Age and sex distribution and heart volume in relation to type of electrode*

	No of pats		Mean age	Heart volume (ml/m² BSA)	
Electrode	Female	Male	(y)	Female	Male
A	5	17	74	480	615
D	12	20	74	495	640
K	14	28	74	550	560
Total	31	65			

Table III *ECG findings in the 94 patients with 96 electrodes*

+S=with syncope −S=without syncope

	AV block II and III		Tachy or brady arrhythmias		Sinoatrial block	
Electrode	+S	−S	+S	−S	+S	−S
A	11	5	4	1	1	0
D	17	10	2	1	2	0
K	24	12	4	1	1	0
Total	52	27	10	3	4	0

Table IV *Stimulation thresholds (voltage pulse form) in relation to pulse width and time after electrode insertion (±S E M)*

Pulse width (msec)	Days after inser tion	Electrode A (n=20)	D (n=31)	K (n=42)	p A D or K	D K
	0	1 0±0 1	0 6±0 03	0 6±0 1	***	N S
	3	2 0±0 2	0 9±0 1	0 9±0 1	*	N S
2 0	7	3 5±0 3	1 7±0 1	1 6±0 1	***	**
	14	3 6±0 3	2 0±0 2	1 6±0 1	*	* *
	30	3 2±0 3	2 0±0 2	1 7±0 1	***	***
	0	1 0±0 1	0 6±0 03	0 7±0 1	***	**
	3	2 1±0 2	1 0±0 1	1 0±0 1	***	N S
1 0	7	3 7±0 4	1 8±0 1	1 7±0 1	***	*
	14	3 8±0 3	2 1±0 2	1 7±0 1	***	***
	30	3 4±0 3	2 2±0 2	1 8±0 1	***	***
	0	1 1±0 1	0 6±0 03	0 7±0 1	*	***
	3	2 2±0 2	1 0±0 1	1 0±0 1	***	N S
0 75	7	3 8±0 4	1 9±0 1	1 8±0 1	* *	
	14	3 9±0 4	2 2±0 2	1 8±0 1	***	***
	30	3 6±0 4	2 3±0 2	1 9±0 2	***	***
	0	1 2±0 1	0 7±0 1	0 7±0 1	***	N S
	3	2 4±0 2	1 1±0 1	1 1±0 1	***	N S
0 5	7	4 2±0 4	2 1±0 1	2 0±0 1	***	*
	14	4 3±0 4	2 5±0 2	2 0±0 2	* *	*
	30	3 9±0 4	2 5±0 2	2 1±0 2	* *	*
	0	1 5±0 2	0 8±0 1	0 8±0 1	* *	N S
	3	3 1±0 2	1 5±0 1	1 3±0 1	***	***
0 25	7	5 4±0 5	2 8±0 2	2 6±0 2	***	**
	14	5 6±0 5	3 2±0 3	2 5±0 2	* *	*
	30	5 3±0 5	3 4±0 3	2 7±0 2	***	* *

p<0 05 ** p<0 01 * p<0 001
D electrode with lower initial threshold

Table V *Stimulation thresholds (constant current pulse form) in relation to pulse widths and days after electrode insertion (±S E M)*

Pulse width (msec)	Days after inser tion	Electrode A (n=20)	D (n=31)	K (n=42)	p A D or K	D K
	0	1 1±0 2	0 4±0 03	0 5±0 1	*	***
	3	2 6±0 3	0 8±0 1	0 8±0 1	***	N S
2 0	7	4 4±0 5	1 5±0 1	1 4±0 1	***	**
	14	4 4±0 4	1 8±0 1	1 4±0 1	***	* *
	30	3 9±0 4	1 7±0 2	1 4±0 1	* *	***
	0	1 6±0 2	0 5±0 1	0 6±0 1	* *	* **
	3	3 8±0 4	1 2±0 1	1 0±0 1	***	***
1 0	7	6 4±0 7	2 2±0 2	1 9±0 1	***	*
	14	6 6±0 6	2 6±0 2	1 9±0 2	*	*
	30	5 8±0.5	2 4±0 3	2 0±0 2	***	
	0	2 0±0 4	0 6±0 1	0 6±0 1	*	N S
	3	4 6±0.5	1 4±0 1	1 2±0 1	*	*
0 75	7	7 6±0 9	2 6±0 2	2 3±0 2	**	*
	14	7 7±0 7	3 2±0 3	2 3±0 2	* *	**
	30	6 8±0 6	2 9±0 3	2 4±0 2	*	***
	0	2 5±0 4	0 7±0 1	0 7±0 1	***	N S
	3	6 1±0 6	1 8±0 2	1 4±0 1	*	***
0 5	7	10 1±1 1	3 5±0 3	3 0±0 3	***	**
	14	10 2±0 8	4 2±0 4	3 1±0.3	**	* *
	30	9 0±0 8	3 7±0 4	3 1±0 3	* *	***
	0	3 9±0 7	1 2±0 1	1 1±0 1	*	
	3	10 0±1 0	3 3±0 3	2 4±0 2	***	**
0 25	7	16 3±1 7	6 1±0 4	4 9±0 4	* *	* *
	14	16 4±1 4	7 2±0 6	5 3±0 5	**	*
	30	14 4±1 3	6 2±0 6	5 2±0 5	*	

* p<0 01 ** p<0 001
D electrode with significantly lower initial threshold

RESULTS

The A electrode was implanted in 22 patients two of whom had to have their electrodes exchanged for D electrodes before the end of the first postopera tive month They were referred to group D which thus consisted of 32 patients One of the latter had the electrode withdrawn after dislocation and no longer being considered a candidate for long term pacing was excluded from the study Another 7 patients had to have reinsertions of the same electrode due to rises in stimulation thresholds The stimulation thresholds before reoperation were not included in the rest of the investigation Therefore the stimulation thresholds reported in Tables IV and V refer to 20 31 and 42 patients for the A D and K electrodes respectively all of which were finally connected to a subcutaneously implanted pulse generator after at least 30 days of uninter rupted function

Irrespective of pulse width and form mean stimu lation thresholds during the first month after inser tion were significantly higher for the A electrode than for the D and K electrodes (p<0 001) (Tables IV and V) With a voltage pulse of 1 msec the maximum mean thresholds of 3 8 and 2 1 V oc curred two weeks after insertion of A and D elec trodes whereas the stable threshold of 1 7 V for the K electrode was reached already after one week (Fig 2) A corresponding stimulation threshold de velopment was found when constant current pulse was used the mean values being 6 6 2 6 and 1 9 mA respectively (Fig 3)

Comparisons between stimulation threshold de velopment in the two small surface electrodes are presented in Tables IV and V It is evident that irrespective of pulse form the stimulation threshold is significantly lower for the K electrode at all pulse widths from the 7th postoperative day When the

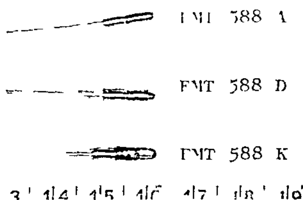


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K	24	12	4	1	1	0
Total	52	27	10	3	4	0

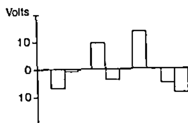


Fig 6 Differences between stimulation threshold values after one month and after 24 months in 10 patients provided with the K electrode

initial stimulation threshold values heart volumes indications for cardiac pacing or age

No infections resulted from the two stage operations either during the first month with external pacemakers or after the subcutaneous implantation of the pulse generator No cardiac perforations occurred and no stimulation electrodes broke

DISCUSSION

In order to prolong battery life it is important to stimulate the heart with the least possible current consumption This can be achieved by decreasing the width and/or the amplitude of the pacemaker impulse

It has been known since long that small surface electrodes give lower thresholds than those with a large electrically active surface (10) Few reports have hitherto been published on long term follow ups of small surface electrodes or on the threshold evolution with different types of electrodes during the first month after electrode insertion and until the stimulation threshold has stabilized (23) In order to find an optimal pacing system it is desirable to compare not only voltage and current thresholds but also such impulse characteristics as duration and output capacitance Some such data have been published (4 7 31) As every pacemaker electrode has its own characteristics it is important to make the above mentioned measurements when introducing a new electrode (9) The measurements should be made with the electronics used in the pacemaker for which the electrode is designed In the present study 3 electrodes with different designs and active surface areas were used thus making it possible to draw general conclusions

It is well known that stimulation thresholds are dependent on many physiological factors (21 27 30) and therefore they were measured in each patient at the same time of day and under as identical circumstances as possible When inexplicable threshold rises were encountered the serum electrolytes were checked but no abnormal values were disclosed Heart volumes differed among the groups and also between men and women within the same group As comparisons in threshold development between men and women in each group revealed no difference of significance heart volume seems to be of no importance for the results This is in accordance with the findings reported by Westerholm (30)

The present investigation in accordance with findings by others (10) confirms that small active surface electrodes give lower energy thresholds than larger ones probably because of higher current density This was true not only immediately after insertion of the electrodes but also throughout the first postoperative month Stimulation thresholds with both large and small conventional surface electrodes increased during the first two postoperative weeks after which they diminished to stable values The new K electrode reached its stable values already after one week although the mean stimulation threshold was insignificantly higher 30 days after insertion However preliminary results from threshold measurements performed two years after electrode insertion in 10 of the 42 group K patients do not show further threshold increase during this time (Fig 6) Compared with the 47 mm² A electrode the maximum mean voltage threshold of the 6 mm² D electrode was about 56% and of the open cage K electrode about 44% When pulse durations were diminished from 2 to 0 5 msec voltage thresholds increased by about 20% irrespective of electrode surface size This can be counterbalanced by an increase in output capacitance (6)

When large capacitors are used more current is consumed by all electrodes but proportionately less by the small surface ones and even markedly less by those with short pulse durations (6) It is therefore obvious that optimal battery life can be obtained with short impulse duration and small surface electrodes (25) Decreasing pulse widths cause increasing stimulation thresholds and it is therefore important to bear in mind that the stimulation threshold can vary by up to 50% from moment to moment dependent on physiological factors (21 27 30) A broad safety margin between the amplitude of the pacemaker impulse and the sti

tion threshold is thus necessary in order to avoid exit blocks Some sudden and inexplicable deaths reported among pacemaker treated patients have supposedly been caused by the absence of this safety margin (13)

The position of the small surface electrode is of vital importance due to the small contact area with the endocardium Feldman and Kantrowitz (8) suggested that low thresholds depend upon direct stimulation of conductive tissue Thus a small change of the electrode position may cause a considerable increase in the stimulation threshold If for some reason the myocardial tissue in that area is or becomes less excitable or the electrode is unduly removed from the myocardium the advantages of the small surface will be lost However only 1 out of 32 conventional small surface electrodes showed an abnormal rise in thresholds during the first postoperative month necessitating reinsertion This was less frequent than with the conventional large surface electrode

The new cage electrode (K) was designed to provide a large contact area while keeping the active surface small No abnormal threshold rise was noticed and it was dislocated in only 2 out of 42 patients It seems that a thrombosis is rapidly formed inside the cage (6) and on becoming organized attaches the cage to the endocardium Furthermore it is likely that the foreign body reaction develops between the chords of the cage and is limits the risk of dislocation of the electrode excitable myocardial tissue The small increase in stimulation threshold indicating a maintained high current density to the myocardium supports this theory It remains to be seen whether these thresholds will remain low at the time of exchange of batteries but hitherto no exit blocks or displacements have occurred during an observation period exceeding 2 years

ACKNOWLEDGEMENT

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Renin-dependent Hypertension in Patients with Unilateral Kidney Disease not Caused by Renal Artery Stenosis

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ABSTRACT The practical value of renin secretion studies in hypertension associated with unilateral kidney disease other than renal artery stenosis, has not been documented This study, comprising 19 patients of this kind, disclosed three who had an abnormal renin secretion from the diseased kidney The level of peripheral renin under basal conditions, and the change from this level as a result of provocation of renin secretion were used to evaluate the importance of an arteriovenous renin gradient in the diseased kidney The three patients were the only ones to become normotensive when the diseased kidney was removed in seven of the cases studied When nephrectomy is considered in severe hypertension with unilateral kidney disease there is a place for renin secretion studies, but a screening procedure is advisable Measuring peripheral renin under basal conditions and after provocation of renin secretion, should reveal whether the renin–angiotensin system might be playing a part in maintaining the high BP The finding of diminishing kidney function in many of the patients, despite good BP control, emphasizes the importance of sparing kidney function whenever possible

In Homer Smith s classical review of 1956 (18) the success rate in curing hypertension by removing a unilateral diseased kidney was 26% Yates Bell (20) claimed a success rate of 38% using methods of selection which included split renal function studies McDonald (16) reported an extremely good cure rate using the Stamey s split renal function tests but it seems well established that well over 50% of patients will be exposed to an unnecessary operative risk if small kidneys of all types are removed These patients will also be deprived of some renal function which may be of substantial importance if the contralateral kidney for one reason or another is lost A refined selective instrument is therefore badly needed in this situation

In the diagnosis of renovascular hypertension renal venous renin studies are well established in the preoperative investigation procedure Renin studies in the preoperative work up of patients with hypertension and unilateral kidney disease have up to now been disappointing as only sporadic cases of abnormal renin secretion have been described in such patients (1 4 6 7 11 12 14 19) It is therefore often inferred that this type of hypertension is not renin-dependent

However it may well be that the renin–angiotensin system plays an essential role in causing the hypertension not only in renovascular hypertension but also in kidneys where contraction or pyelonephritic endarteritis is creating ischaemic zones (15) Contracted kidneys in dialysis patients with very severe hypertension are also known to produce great amounts of renin (10) Therefore in our consecutive series of hypertensive patients subjected to renin secretion studies performed since 1970 we have also included many patients with unilateral small kidneys The aim of this investigation is to find out whether this routine has contributed favourably to the treatment

PATIENTS

From our consecutive series of hypertensive patients investigated during 1970–75 19 patients were included in this study 8 males with a mean age of 50 years (range 41–64) and 11 females with a mean age of 33 5 years (range

Table I. *Review of the 19 patients*

GFR=glomerular filtration rate. FH=fundus hypertonicus

Pat no	Sex	Age (y)	Diagnosis	Sign or symptoms of infection	Total GFR (ml/min/1.73 m²)	Function on affected side (%)	FH	Treatment*
10	♂	50	Hypoplasia	No	96	<10	II	H+M
13	♀	32	Hypoplasia	Yes	96	20	II	BB+H
17	♀	32	Hypoplasia	No	75	25	0	H+SB
18	♀	55	Dysplasia	No	81	40	II	M+S
27	♂	60	Papillitis necroticans	No	72	<10	II	None
123	♀	27	Hypoplasia	Yes	59	<10	III	BB+H
128	♀	25	Hypoplasia	No	100	<10	0	BB+H
136	♀	44	Pyelonephritis	Yes	97	<10	III	S
138	♀	21	Pyelonephritis	Yes	92	35	III	BB+C+H+SB
140	♂	55	Hypoplasia	No	52	<10	II	BB+H
141	♂	64	Status post hydronephrosis op	No	60	<10	–	BB+H
146	♀	30	Hypoplasia	Yes	33	<10	II	BB+H
147	♂	58	Hypoplasia	No	86	15	0	H+SB
156	♂	46	Status post hydronephrosis	No	Cr/s 1.1 *mg/100 ml*	<10	I	BB
201	♂	24	Pyelonephritis calculosa	Yes	101	35	I	BB
202	♂	41	Status post hydronephrosis op	No	80	<10	II	H+SB
204	♀	34	Pyelonephritis	Yes	41	<10	–	None
213	♀	46	Pyelonephritis*	Yes	96	<10	II	A+BB+H+S
219	♀	26	Status post hydronephrosis op	Yes	75	<10	III	BB+S

* Preoperative

H=hydralazine. M=methyldopa. BB=β-blocker. SB=sympathetic blocker. S=diuretic. C=clonidine. A=spironolactone

21–55). Only patients with unilateral kidney disease not caused by renovascular disease have been included. A review of the patients is given in Table I. All but one have been followed for more than a year after the operation or after the renin studies. Patient 213 died 4 months after the operation in a cerebrovascular accident. The initial investigation was performed during the patients' hospitalization and the follow-up studies were performed at the Out-patient Department.

METHODS

Procedure

The patients were admitted to hospital and, whenever possible, taken off treatment 3–4 days prior to the renin secretion study. Longer periods off treatment were exceptional and not considered justified if not carried out under close observation. The patients were placed on a regular hospital diet. The renin secretion studies were performed between 8 and 12 a.m. Catheters were inserted into a brachial artery or brachial vein and into the two renal veins, in the latter case by the transfemoral route. Correct positioning of these catheters was ascertained by X-ray, oxygen measurements and/or the extraction ratio of PAH. The AV difference of renin was measured by drawing simultaneous blood samples in the three catheters under basal conditions and after provoking renin secretion either by pharmacological means (i.v. injection of dihydralazine 7.5 mg or furosemide 40 mg) or head-up tilting. The second set of samples was taken 30 min after the dihydralazine injection, one hour after the furosemide injection or after 10 min tilting.

Renin measurements

Plasma renin activity (PRA) was measured using bioassay (pats 10–147) or radioimmunoassay (pats 156–219).

Bioassay of PRA was performed using the method of Boucher et al (8, 9). Disodium EDTA was used as anticoagulant and a three-hour incubation period was allowed. Angiotensin was estimated by the BP method in a 4-point assay on pentolinium-blocked nephrectomized rats under barbiturate anaesthesia with electromano-

Table II *Results of renin secretion studies on operated patients*

PRA=plasma renin activity RRV=right renal vein LRV=left renal vein T=tilt F=furosemide H=dihydralazine +=positive −=negative A=ambiguous

Pat no	Affected side	Procedure	PRA Peripheral	RRV	LRV	Conclusion
123	L	Basal	1 121	500	1 664	+
		F	901	1 155	1 547	
136	R	Basal	120	98	91	A
		F	83	184*	72	
138	R	Basal	222	794*	741	+
		F	751	1 135	806	
140	R	Basal	103	157	132	−
		F	126	149	67	
202	R	Basal	1.4	1.4	1.1	−
		H	1.5	1.4	1.2	
213	L	Basal	10.4	10.4	15.3	+
		H	16.5	16.7	47.3*	
219	R	Basal	1.7	3.9*	2.3	A
		H	2.5	4.0*	2.1	

Significant difference from corresponding peripheral value in the case of renal veins significant difference from peripheral value under basal conditions in the case of peripheral stimulated value

metric recording of the mean carotid pressure Synthetic VAL 5 angiotensin (CIBA) was used as standard The error of a single determination is ±20% and the recovery of added angiotensin to the extraction procedure is 75% The normal range of PRA is 100–400 ng angiotensin/100 ml/3 h

The radioimmunoassay of renin in our laboratory has been described in detail (5) the assay for angiotensin I as described by Giese et al (13) being used Inactivation of angiotensinases is achieved by dialysis of the plasma sample After incubation angiotensin I is extracted by means of SP sephadex and the eluate used for the assay Separation of the bound fraction is achieved with G 25 sephadex The error of single measurements is 14% and the normal range for PRA 0.3–2.0 ng ml⁻¹ h⁻¹ The significant correlation between the two methods in our laboratory is given by the equation $Y=0.75X+0.16$ where Y is the result of bioassay and X the result of radioimmunoassay (both given in ng AT I ml⁻¹ h⁻¹ $r=0.87$ $n=48$) The values in bioassay will then be approximately 25% lower than those in radioimmunoassay

Estimation of individual kidney function

The blood flow of each kidney is estimated from isotope renograms the determinations being performed essentially as described by Pedersen and Poulsen (17) and Aurell et al (3)

The isotope activity over each kidney was determined 2 min after the injection of 20 μCi 131 hippuran and the fractional share of the renal function is thereby easily determined Total renal function was determined by total renal clearance of inulin or 51-chrom-EDTA By combining these two measurements the function of each kidney could be assessed

Classification of results

The patients are referred to one of three groups 1) *Renin positive* Patients having renin levels above the normal range in peripheral blood under basal conditions and/or a significant rise of renin in peripheral blood as the result of a provocation procedure and whose diseased kidney was shown to be the sole source of the abnormal renin secretion 2) *Renin-ambiguous* Patients having renin within normal limits both under basal conditions and after provocation and no significant change by the provocation procedure but having a significant venoarterial difference of renin over the diseased kidney 3) *Renin negative* The remaining patients

Tests for statistically significant differences have been carried out according to the formula (12)

$$d=\frac{|PRA_1-PRA_2|}{\text{error}\sqrt{PRA_1^2+PRA_2^2}}$$

requiring $d>1.96$ ($p<0.05$) for acceptance

RESULTS

The results of renin measurements are given in Tables II and III Three patients were classified as positive according to our definitions eight as ambiguous and eight as negative Seven of the 19 patients were operated upon (Table II) the three patients with an abnormal renin secretion two in the ambiguous and two in the negative group Of the

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Table III *Results of renin secretion studies on non operated patients*
Abbreviations and symbols as in Table II

Pat no	Affected side	Procedure	PRA Peripheral	PRA RRV	PRA LRV	Conclusion
10	R	Basal	120	183	137	–
		T	126	179	175	
13	R	Basal	54	104[a]	75	A
		T	73	95	90	
17	R	Basal	18	43[a]	87[a]	–
		T	8	29[a]	39[a]	
18	R	Basal	155	163	153	A
		T	181	191	321[a]	
27	R	Basal	100	100	100	–
		H	107	112	100	
128	R	Basal	419	360	226	–
		F	325	320	173	
141	R	Basal	65	77	58	A
		F	75	145[a]	78	
146	R	Basal	181	334[a]	229	A
		F	215	537[a]	91	
147	L	Basal	91	68	114	A
		F	52	71	131	
156	L	Basal	1 0	1 3	3 1[a]	A
		F	1 2	1 5	3 2	
201	R	Basal	2 8	3 2	3 1	–
		H	2 9	3 8	5 2[a]	
204	L	Basal	0 4	0 5	0 5	–
		H	0 5	0 8[a]	0 6	

[a] See note Table II

tients not operated upon (Table III) six were ambiguous and six negative

Patients who were renin positive achieved normotension postoperatively while the remaining four patients who underwent operations and all the non operated patients had a persisting hypertension

Kidney function was evaluated in the follow up study It was found that the operated renin positive and normotensive patients had the same or increased contralateral kidney function as before the operation One out of four patients with poor BP control escaped reduction of kidney function and six out of 11 with good BP control had preserved renal function

Of the patients not operated upon none had signs or symptoms indicating a malignant or very severe type of hypertension In most of them it was mild and easily controlled On the other hand the operated patients presented several interesting features and will therefore be described in some detail below

CASE HISTORIES

Renin positive patients

Patient 123 A woman aged 27 whose mother has hypertension She has had recurrent urinary tract infections since early childhood A slight elevation of BP was first observed when she was 25 years of age When examined a year later hypertension of 180/130 mmHg was observed and severe hypertensive eye ground changes (FH III) were found Once treatment was instituted her BP was easily controlled Her left kidney was found to be very small (3 5×7 5 cm) with a hypoplastic pelvis The right kidney was slightly hypertrophied (7×13 cm) and without pathological findings on X ray There was no ureteral reflux or renal artery stenosis Concentrating capacity tested by pitressin tannate was only 545 mOsm/kg glomerular filtration rate (GFR) was reduced (Table I) These two findings indicate that the function of the right kidney also was impaired An abnormal renin secretion was found from the small kidney (Table II) Nephrectomy of a 7×4 cm kidney was performed Examination revealed both

hypoplas a and pyelonephritic changes After interrup on of antihypertensive treatment postoperatively normoten s on has pers sted for 2½ years and GFR has improved to 75 ml/m n/1 73 m² BSA

Pat ent 138 A woman aged 71 with no family h story of hypertens on At the age of 70 she suffered from head ache and ep stax s She was found to have a BP of 220/145 mmHg and severe hypertens ve eye ground changes (FH III) A year prev ously she had been normotens ve Investigation now revealed a small kidney (5×12 cm) on the right s de without characterist c s gns of hypoplas a The contralateral k dney was large (8×15.5 cm) and showed no rad ological s gns of d sease Kidney function on the affected s de was one th rd of total (Table I) Urinary cultures were pos tive but there was no prev ous h story of urinary tract infect ons Desp te the f nd ng of an abnormal ren n secret on (Table III) nephrectomy was postponed because of the important contribut on to total k dney funct on from the right s de Nephrectomy was performed when it was found that BP control was poor de sp te a 4-drug regimen After interruption of ant hyperten s ve treatment postoperat vely she has rema ned normo tens ve for 2 years GFR in the remain ng kidney has not mproved ind cat ng that no compensatory hypertrophy has taken place This may be a s gn that the remaining k d ney was affl cted too

Pat ent 213 A woman aged 46 w th no fam ly h story of hypertens on She had a h story of urinary tract nfec t ons and urinary stones and known hypertens on for at least 10 years previously She suffered an attack of pul monary oedema at the age of 43 and was then found to have a BP of 280/180 mmHg During the next few years an mpress ve secondary hyperaldosteronism was discov ered but her BP was eas ly controlled It was found that the left kidney had shrunk from a normal size 6 years prev ously to 9×5 cm and it showed scarring There was no renal artery stenosis or obstruction to urinary flow About one f fth of k dney funct on was found on the left side *(Table I) The right k dney was large (13 5×6 cm)* and showed no rad ological s gns of d ease The ren n secre tion from the left k dney was very h gh and nephrectomy was performed The k dney we ghed only 60 g The paren chyma d d not show signs of pyelonephrit s The intrare nal vessels were markedly hypertroph ed and arterioscle rot c Normotension was establ shed mmed ately and persisted for 4 months unt l a thrombos s of the bas la artery ended her l fe

Ren n-amb guous or nega e pat ents

Pat ent 136 A woman aged 44 w th no fam ly h story of hypertens on *Repeated urinary tract infections Hyper* tension was d scovered one year prior to nvest ga on D abetes mell tus was d scovered shortly before nvest ga t on Her opt c fundi showed severe changes class f able as FH III A BP of 210/130 mmHg was recorded A small k dney (8×4 5 cm) was d scovered on the left s de and t was respons ble for less than 10% of k dney funct n wh ch was with n the normal range The right k dney showed no s gns of d sease and was large (19×10 cm) Results of ren n stud es were amb guous and nephrecto-my was cons dered worth a trial as the pat ent s BP was somewhat d ff cult to keep under p oper control The op-

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came normotensive after nephrectomy of the small kidney This may indicate that the frequency of renin-dependent hypertension suitable for surgical treatment by nephrectomy in patients with unilateral kidney disease of non renovascular origin is only about one of six This low figure, lower than one of four reported by Smith (18) may be due to our careful exclusion of stenotic lesions of the renal artery as the cause of kidney contraction Nevertheless as our case histories indicate, one of the three patients (no 213) had a puzzling unilateral kidney contraction where disseminated intrarenal vascular lesions seem to have played an important role

The case histories also show that the hypertension in the three renin-dependent cases was of very severe type and that the clinical conditions were rather alarming In this kind of situation it is natural to proceed with investigations to reveal an underlying cause of the elevated BP Our findings show that it is in this group of patients with unilateral kidney disease that renin studies may be rewarding but that there may be a need for a screening procedure This need is emphasized by the fact that renal vein catheterization in these patients is difficult as renal veins can be very small and the blood flow through the kidney very reduced giving rise to sampling difficulties

The finding of a high peripheral renin value under basal conditions warrants further investigations As by one of our patients (no 138) this may not be reliable as a sole screening procedure kind of provocation of renin secretion should therefore be carried out as well a significant rise in the renin level being taken as a sign that the renin-angiotensin system is playing a role in maintaining hypertension

The interpretation of the ratios and numerical differences between renin concentrations or activities in renal veins and peripheral vessels is often difficult The plasma flow through the diseased kidney is much reduced and true flow values are not easily obtained The calculations of the individual renin secretion rates are therefore fallacious as the likely errors in the renal plasma flow measurements will greatly affect the results The finding of a high or rising renin concentration in peripheral blood is a logical sign of abnormal renin secretion which can be used to interpret the importance of the renin gradient over the diseased kidney

The possibility cannot be ruled out that some patients without demonstrable renin secretion may benefit from nephrectomy though very rarely mechanism of hypertension in such cases rem poorly understood The hypertension in the ca not cured by nephrectomy and where no abnorm ity of renin secretion can be demonstrated can ther be of the essential type or may originally h been caused by the diseased kidney The hyper sive process in the latter case could then h reached the stage where changes have taken pl in the contralateral kidney, which may then be sponsible for the high BP

Finally the observation of a deteriorating re function in half of the patients showing good control at follow up may be important It certai indicates that the renal disease is progressive many cases and it underlines the importance sparing renal function and refraining from nephr tomy whenever possible

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Albumin Metabolism and Gastrointestinal Loss of Protein in Chronic Renal Failure

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ABSTRACT The catabolism of albumin labelled with ^{125}I has been studied in 10 patients with advanced renal failure and in 5 with nephrotic syndrome In 10 patients the gastrointestinal protein loss was studied simultaneously by determining the faecal excretion during 7 days of ^{51}Cr after i v administration of ^{51}Cr labelled chromic chloride The results were related to a control group in which 12 subjects were studied with respect to albumin catabolism and 17 with respect to the gastrointestinal protein losses The results showed that 1) In the two patient groups the means for serum albumin concentration and the intravascular albumin pool expressed as g or g/kg b wt , were significantly decreased compared with those of the control group 2) The two patient groups had an increased extravascular albumin pool as well as an elevated ratio between extra and intravascular pools 3) The mean albumin catabolic rate was not increased in the renal insufficiency group expressed as a percentage of the intravascular pool/24 h or as g/24 h In the patients with nephrotic syndrome however, it was significantly increased 4) The renal insufficiency group had a mean cumulative ^{51}Cr excretion during 7 days of 1 6±0 80% of the given dose the control group 0 63±0 30% This difference is highly significant The patients with nephrotic syndrome did not differ from the control group

Patients with advanced chronic renal insufficiency often develop hypoproteinaemia and hypoalbuminaemia in many cases without renal protein losses There are many conceivable causes of this condition protein depletion secondary to prolonged anorexia and restriction of protein in the diet (1 10 12) abnormal distribution (4) a defective synthesis of proteins (6 12) or combinations of causes

A general vascular damage by which protein is lost into extravascular spaces might also cause protein losses into e g the gastrointestinal tract This was indicated on a small scale in another disease with generally occurring vascular lesion i e rheumatoid arthritis (16) In the present study this mechanism has been evaluated simultaneously with the albumin metabolism in patients with advanced uraemia and in patients with nephrotic syndrome as well as in a control group

MATERIAL

Sixteen healthy persons with normal haematology and serum protein patterns 12 females and 4 males age 20–51 years This group has been presented in detail elsewhere (16) *Ten patients with advanced renal failure* 3 females and 7 males age 24–62 years (Table I) *Five patients with nephrotic syndrome* 4 without uraemia and one with moderate uraemia age 43–62 years (Table II) For comparison *4 patients with renal insufficiency treated with weekly peritoneal dialysis* were analyzed with respect to the albumin balance during 5–8 months They were all on a diet with protein restricted to 40 g daily Informed consent to the study was given by all subjects

METHODS

The methods for studying ^{125}I albumin catabolism and the cumulative 7-day faecal excretion of ^{51}Cr have recently been described in detail (16) Briefly ^{125}I labelled albumin was given i v Blood samples were taken after 10 min and once daily throughout the first 2 weeks and then every second day All urine was collected in 24 hour batches throughout the study The plasma and whole blood samples drawn 10 min after the injection of ^{125}I albumin were used for blood and plasma volume determination according to isotope dilution principles The retained dose was obtained by summing the urinary excretion of ^{125}I and subtracting from the given dose The size of the ex

Table III *Laboratory data and results*

Case no	Sex	Age (y)	B wt (kg)	Serum creatinine (mg/100 ml)	Urinary protein (g/24 h)	Albumin: Serum conc (g/100 ml)	Albumin: Blood volume (l)	Albumin: IV pool g	Albumin: IV pool g/kg b wt
Renal failure									
1	♂	38	59	11 3	1 3	3 9	6 7	169	2 85
2	♂	48	88	11 8	1 5	2 8	6 8	160	1 8[illegible]
3	♂	53	68	9 5	2 1	3 1	6 9	137	2 02
4	♂	48	69	5 1	2 4	3 4	4 0	94	1 37
5	♂	31	68	6 1	4 6	4 5	5 3	139	2 06
6	♂	56	82	13.5	5 2	3 0	6 1	142	1 73
7	♀	28	59	9 2	2 2	3 2	5 1	131	2 23
8	♀	44	54	11 8	3 8	3 4	4 7	107	1 98
9	♂	24	62	12.5	1 6	2 7	5 4	115	1 87
10	♂	57	54	3 2	0 9	3 9	4 5	117	2 17
Nephrotic syndrome									
11	♂	43	80	1 1	11 6	2 1	5 5	79	0 98
12	♀	62	56	8 2	16 8	1 7	4 0	47	0 85
13	♀	47	64	1 3	7 7	2 9	3 4	61	0 97
14	♂	49	84	1 0	10 3	2 9	6 3	113	1.35
15	♀	62	47	0 6	5 3	1 9	3 6	46	0 97

more protein restriction has been found to lead to decreased albumin metabolism (12) This might explain the extremely low catabolic rate in three patients in a final stage They had a long history of the illness during which their protein intake had been limited both by a low protein diet and by anorexia ... isone has been shown to increase albumin ... (7 19) Some of our patients were ... l with cortisone (Table I) but their albumin metabolism did not differ from the rest of the grou (Table III) The possible influence of cortison treatment is probably less important than othe factors regulating albumin metabolism in uraem patients In the uraemic patients urinary protei loss did not influence the IV albumin pool/kg to great extent in contrast to the patients with ne phrotic syndrome

Faecal loss of protein was increased in th

Table IV *Comparisons between the controls and the patient groups*

	Controls (n=16) Mean	Controls (n=16) S D	Renal insufficiency (n=10) Mean	Renal insufficiency (n=10) S D	Renal insufficiency (n=10) t value	Nephrotic syndrome (n=5) Mean	Nephrotic syndrome (n=5) S D	Nephrotic syndrome (n=5) t value
Serum creatinine (mg/100 ml)			9 4	3 5		2 4	3 2	
Urinary protein loss (g/24 h)			2 6	1 5		10 3	4 4	
Serum albumin conc (g/100 ml)	5 1	0 28	3 4	0 56	10 332***	2 3	0 57	15 10
IV albumin pool (g)	148	27 8	131	23	1 625n.s.	69	28	5.545 *
IV albumin pool (g/kg)	2 47	0 28	2 0	0 38	3 553 *	1 02	0 19	10 735
EV/IV ratio	1 16	0 15	2 8	1 23	5 335 **	3 6	2 8	3 687
Albumin catabolism % of IV/24 h	7 82	0 99	5 6	4 1	1 744 n.s.	26 4	6 2	10 078
g/24 h	11 4	2 43	7 4	5.8	2 076*	18 1	7 3	2 833
Cumulative excretion of ^{51}Cr in faeces	0 63	0 30	1 6	0 80	4 210 **	0 78	0 16	0 937 n.s.

n.s.=not significant *0 01<p<0 05 ** 0 001<p<0 01 ***p<0 001

EV pool (g)	EV/IV	Catabolism % of IV pool	Catabolism g/24 h	T½ (d)	^{51}Cr in faeces (%/7 d)
691	4 09	11 3	19 1	15 2	–
637	3 98	0 9	1 5	21 8	–
701	1 47	3 5	4 9	26 9	–
238	2 53	11 3	10 6	13 4	1 22
279	2 01	7 3	10 1	21 7	–
276	1 59	8 0	11 4	12 4	0 78
634	4 84	1 8	2 4	12 4	2 05
741	2 25	2 4	2 5	12 6	3 01
417	3 62	1 0	1 1	14 9	1 41
188	1 61	8 6	10 1	18 1	1 10
658	8 33	26 7	21 1	4 1	–
186	3 96	19 6	9 2	7 4	0 68
68	1 11	24 0	14 6	5 7	1 00
263	2 35	25 4	28 7	6 0	0 64
107	2 32	36 3	16 7	1 9	0 80

uraemic patients The pathogenesis is unknown It might be a symptom of the uraemic enteropathy or a sign of increased general vascular permeability Increased loss of protein from the gastrointestinal tract has been shown to occur e g in rheumatoid arthritis (16) and in diabetes mellitus (14) In the present group the protein loss was smaller than in diabetic patients and in patients with gastroenteropathies Thus in 20 patients with gastroenteropathies we found a mean 7-day cumulative ^{51}Cr faecal excretion of 7 5±5 5% of the given dose of ^{51}Cr In uraemic patients this comparatively small increase in the faecal protein loss might be of importance for the IV albumin pool considering that many patients have a depressed albumin metabolism due to e g protein depletion Faecal protein loss was not increased in the patients with nephrotic syndrome which is consistent with the findings by Jensen et al (11) Thus urinary and faecal losses are not very big Peritoneal dialysis however is known to cause considerable protein loss Our figures for protein loss with dialysis fluid in 4 patients compare well with those of Berlyne et al (3) This protein loss was only partly compensated for by blood and/or albumin transfusions

Table V *Protein losses in four patients undergoing regular peritoneal dialysis*

Case no	Diagnosis	Month (no)	Albumin loss with dialysate (g)	Cumulative albumin balance (g)	Protein loss with urine (g)	Serum albumin (g/100 ml)
16	Chronic glomerulonephritis +nephrosclerosis	1	132	+ 48	60	3 2
		2	180	+ 28	36	3 3
		3	200	−112	30	3 5
		4	200	−232		3 4
		5	200	−412		3 4
17	Necrotizing papillitis +chronic pyelonephritis	1	200	− 80	–	3 4
		2	132	−232		3 5
		3	200	−332		3 5
		4	208	−440		3 9
		5	270	−580		3 6
		6	200	−720		3 1
		7	200	−860		2 9
		8	210	−930		2 8
18	Chronic pyelonephritis	1	50	+ 70	126	4 3
		2	174	− 24	69	3 9
		3	120	+ 16	33	3 7
		4	120	− 64	33	4 0
		5	125	−150	18	3 7
19	Chronic glomerulonephritis +nephrosclerosis	1	134	− 74	102	4 0
		2	190	−224	72	4 1
		3	206	−410	27	3 9
		4	180	−530	21	3 8
		5	248	−698	21	3 3
		6	210	−900	0	3 0

Table I *Average urinary protein excretion (mg/24 h) in 22 patients during the febrile period and 221 control subjects and number of observations above the reference interval of the controls*

	Patients		Controls		Observations above the reference interval	
	Median	Interval	Median	Reference interval	%	*n*
Albumin	25*	4–914	6 2	1 6–34 2	32	7
Transferrin	3 8*	0 2–63 6	0 7	0 0–3 5	50	11
Haptoglobin	2 4*	0 1–6 9	0 1	0 0–1 0	82	18
IgG	8 9*	1 0–35 9	1 9	0 2–6 5	73	16
IgA	4 8*	0 8–14 7	0 6	0 0–2 3	82	18
IgM	2 4*	0 0–18 0	0 3	0 0–1 3	64	14
Lambda	6 3*	0 0–18 6	0 8	0 0–2 4	86	19
Kappa	14 0*	0 0–55 6	1 2	0 0–6 8	86	19
Lysozyme	0 29*	0 00–0 96	0 16	0 02–0 45	41	9
β_2 microglobulin	0 20*	0 02–1 57	0 04	0 00–0 14	73	16

* $p<0.001$

patient peritonsillar abscesses in 2 patients cholecystitis in 2 and influenza in 1 patient In 2 patients the cause of fever was unknown The patients had neither previous or present nephro-urological diseases nor generalized dis orders which might involve the kidneys None of the patients had malignant diseases congestive heart failure epilepsia or other convulsions Patients with indwelling bladder catheter were excluded BP was 140/90 mmHg or less in patients under 60 years and 160/90 mmHg or less in patients aged 60 years or more All patients had normal serum creatinine concentration and normal creatinine clearance related to body surface age and sex (14)

In each patient 24 hour urines were collected from the onset of fever in 10 patients continuously every day until [illegible] rge from hospital or from the unit because of opera [illegible] treatment Venous blood was drawn every second or [illegible] day The samples were kept below +4 C without addition of preservatives and the protein analysis was carried out within 5 days after collection The urinary sediment showed no erythrocytes leucocytes or casts and the pH of all urine samples was above 5 5 None of the patients had bacteriuria or other signs of urinary tract infection

The urinary and serum creatinine concentrations were determined by Jaffe s reaction (AutoAnalyzer®) The total urinary protein concentration was determined by a tetra bromphenolblue reaction (8) Specific proteins in urine and serum were determined on AutoAnalyzer® (Techni con Corp Tarrytown N Y) The method used was an immunoprecipitin reaction The precipitate was measured by fluoronephelometry Monospecific antisera against the high molecular weight proteins were Technicon products and monospecific antisera against lysozyme and β_2-microglobulin were Dakopatts products The mono-specific antisera against lambda and kappa light chains from the immunoglobulins were absorbed goat anti-sera (Behringwerke) which reacted only with the free light chains

In order to increase the sensitivity the standard method was modified as described previously (7 12) The average

Table II *Average urinary protein excretion (mg/24 h) in the febrile periods with average body temperatures ≥38 5 C and <38 5°C*

	Body temperature ≥38 5 C (*n*=11)		Body temperature <38 5 C (*n*=11)	
	Median	Interval	Median	Interval
Albumin	28	9–203	22	4–914
Transferrin	5 7	0 3–22 1	2 7	0 2–63 6
Haptoglobin	3 0	0 7–6 9	1 7	0 1–6 0
IgG	10 4	5 9–30 6	7 5	1 0–35 9
IgA	7 7	1 [illegible]–14 5	3 8	0 8–14 7
IgM	2 8	0 [illegible]–18 0	1 6	0 0–14 0
Lambda	8 0*	2 1–16 0	3 4	0 0–18 6
Kappa	25 3*	3 8–50 4	9 6	0 0–55 6
Lysozyme	0 52*	0 02–0 83	0 22	0 00–0 96
β microglobulin	0 44 *	0 15–1 57	0 10	0 02–0 46

$p<0.05$ * $p<0.01$

Table III *Urinary glomerular protein excretion in 10 febrile patients*

Three values for each patient are included febrile course 3rd day after disappearance of fever last day of observation expressed as the relative clearance (1×10^{-6})

Pat no	Albumin	Transferrin	Haptoglobin	IgG	IgA	IgM	Relative frequency*
1	181	189	11	17	35	6.8	5/6 +
	4.2	21	4.7	5.4	22	15	3/6 +
	4.2	21	5.5	5.2	21	14	4/6 +
2	64	97	13	22	23	102	6/6 +
	3.0	9.1	4.2	5.2	5.9	31	3/6 +
	2.7	11	9.4	6.7	6.7	35	4/6 +
3	23	78	35	34	88	219	6/6 +
	9.1	23	21	8.2	40	197	6/6 +
	4.6	7.7	0.0	0.0	4.0	24	2/6 0
4	31	47	26	42	125	52	6/6 +
	52	71	24	47	130	46	6/6 +
	48	67	22	42	46	114	6/6 +
5	6.5	13	0.5	4.5	8.2	6.1	3/6 +
	3.4	11	1.0	3.5	5.9	6.4	1/6 0
	5.0	6.6	1.2	3.0	1.6	13	0/6 0
6	1.6	3.8	8.2	3.9	6.3	6.7	1/6 0
	1.7	3.4	7.1	3.1	4.9	5.6	1/6 0
	2.0	3.6	7.5	3.5	6.8	19	2/6 0
7	5.4	13	5.2	10	19	43	4/6 +
	2.4	11	1.7	9.0	17	32	4/6 +
	2.2	4.3	2.5	4.3	9.6	23	2/6 0
8	6.6	13	28	9.4	9.8	11	5/6 +
	2.8	6.0	9.7	4.2	4.8	13	1/6 0
	2.6	4.5	7.8	2.8	6.7	22	2/6 0
9	4.7	9.0	17	7.9	23	36	5/6 +
	4.6	35	18	14	42	59	5/6 +
	4.1	22	23	12	36	56	5/6 +
10	5.9	10	0.2	4.1	4.4	0.0	1/6 0
	3.5	1.8	0.0	1.5	0.9	12	0/6 0
	3.2	4.5	0.6	1.3	1.9	0.0	0/6 0
Ref *	6.3	7.0	5.2	4.6	7.0	15.0	

Relative frequency of values above the upper limit (+ = glomerular type of proteinuria 0 = insignificant protein excretion)

*Upper limit of the reference interval

sensitivity was 0.1 mg/l depending on the range of measurement The precision of the method (coefficient of variation) expressed as daily within run and between runs averaged 4.6% and 9.6% respectively *The analytical capacity was 60 specimens per hour*

For sequential studies the relative clearance of the 6 high molecular weight proteins was calculated by the formula

$$\frac{U_p \cdot V/S_p}{U_{cr} \cdot V/S_{cr}}$$

and the relative excretion of the 4 low molecular weight proteins by the formula

$$\frac{U_p \cdot V}{U_{cr} \cdot V/S_{cr}}$$

where U_p and S_p denote the urinary and serum concentration of the specific protein U_{cr} and S_{cr} the urinary and serum concentration of creatinine and V urine volume per unit of time Thus the clearance or excretion was expressed relative to GFR as proposed by Manuel et al (15) and Ravnskov (17)

The non parametric Mann-Whitney test was employed for statistical evaluations We consider $p<0.01$ as significant $0.01<p<0.05$ as possibly significant and $p>0.05$ as insignificant

RESULTS

The urine samples collected from each febrile period (days with body temperature ≥38.0 C) were tested with Albustix A positive protein reaction was demonstrated in the febrile periods of 6 patients

Table IV *Urinary tubular protein excretion in 10 febrile patients*

Three values for each patient are included febrile course 3rd day after disappearance of fever last day of observation expressed as the relative excretion (1×10⁻³)

Pat no	Lambda	Kappa	Lysozyme	β_2 microglobulin	Relative frequency[a]
1	57	155	5 2	2 0	3/4 +
	6 5	12	6 3	1 3	1/4 0
	8 4	11	6 4	1 4	1/4 0
2	96	142	8 7	2 0	4/4 +
	4 0	20	2 7	0 4	0/4 0
	6 0	19	5 1	0 7	1/4 0
3	115	360	11	6 1	4/4 +
	40	88	3 9	2 0	2/4 +
	21	15	0 7	0 5	0/4 0
4	36	150	14	3 3	4/4 +
	7 3	53	7 7	1 6	1/4 0
	16	58	6 8	1 2	1/4 0
5	88	157	0 0	0 5	2/4 +
	21	47	0 0	0 8	0/4 0
	16	46	0 0	0 8	0/4 0
6	93	252	3 0	1 5	2/4 +
	25	159	2 7	0 7	2/4 +
	17	172	3 3	0 8	1/4 0
7	79	196	6 1	2 5	4/4 +
	4 1	45	4 0	0 6	0/4 0
	11	12	2 1	0 6	0/4 0
8	114	572	0 5	11	3/4 +
	23	233	0 5	1 1	1/4 0
	18	143	0 3	1 4	1/4 0
9	109	247	5 9	2 8	4/4 +
	47	154	8 1	2 1	4/4 +
	31	54	9 0	2 1	3/4 +
10	83	132	0 1	1 4	2/4 +
	32	19	0 3	0 3	1/4 0
	13	14	0 0	0 3	0/4 0
Ref[b]	24	66	4 2	1 6	

[a] Relative frequency of values above the upper limit (+=tubular type of proteinuria 0=insignificant protein excretion)
[b] Upper limit of the reference interval

(27%) Total urinary protein excretion exceeded the reference interval in 8 patients (36%) during the febrile periods whereas one or more of the specific proteins exceeded the reference interval in 21 patients (95%)

Table I shows the average 24 hour urinary excretion of 10 specific proteins all significantly increased during the febrile period in 22 patients compared with the control subjects The same significance level was obtained when only febrile periods with Albustix negative urines were compared with the controls On an average the urinary excretion of the high and low molecular weight proteins exceeded the reference interval of the controls in 64% and 72% of the febrile periods respectively Increased excretion of the free lambda and kappa light chains was encountered in 19 febrile periods whereas increased excretion of albumin was demonstrated in 7 febrile periods

Table II shows the average urinary excretion of all protein components during febrile periods with average body temperature ≥38 5 C and <38 5°C The excretion of β_2 microglobulin was significantly increased ($p<0$ 01) in febrile periods with an average body temperature ≥38 5 C while the increased excretion of the free lambda and kappa light chains and lysozyme was possibly significant ($p<0$ 05) The excretion of the other protein components was *not significantly increased*

Sequential studies for 6–13 days were performed in 10 of the 22 febrile patients In these studies a glomerular type of proteinuria was defined as an increased relative clearance of at least 3 of the 6 high molecular weight proteins and a tubular type of proteinuria as an increased relative excretion of at least 2 of the 4 low molecular weight proteins above the upper limit of the reference interval (controls) The relative clearance of the 6 high molecular weight proteins in these 10 patients is given in Table III In 8 of the 10 patients (nos 1–5 7–9) a glomerular type of proteinuria was demonstrated during the febrile period Three days after the fever had subsided the glomerular type of proteinuria persisted in 6 patients (nos 1–4 7 9) and on the last day of observation in 4 (nos 1 2 4 9)

Table IV shows the relative excretion of the 4 low molecular weight proteins A tubular type of *proteinuria was demonstrated during the febrile period* in all 10 patients three days after the fever had subsided in only 3 patients (nos 3 6 9) and on the last day of observation in one patient (no 9) only

DISCUSSION

Febrile diseases were accompanied by a significantly increased urinary excretion of both high and low molecular weight plasma proteins in all but one of our 22 patients as shown in Table I Thus a mixed glomerulo-tubular type of proteinuria was demonstrated in febrile diseases Increased excretion of haptoglobin IgG IgA free lambda and kappa light

chains from immunoglobulin and β_2 microglobulin was the most common finding The excretion of albumin exceeded the reference interval in 7 patients only and 6 urines showed a positive Albustix reaction when tested for protein This finding is in agreement with several earlier studies on the ability of Albustix to detect increased urinary protein excretion (1 2 9 10 13 16)

The glomerular and tubular types of proteinuria appeared to run different courses in febrile patients Tubular proteinuria was significantly increased in patients with a body temperature ≥38 5 C compared with those with <38 5 C whereas the magnitude of glomerular proteinuria was not significantly influenced by the severity of fever (Table II) Furthermore the sequential studies of 10 patients (Tables III and IV) demonstrated that tubular proteinuria occurred in all patients and disappeared within 3 days after normalization of the temperature This finding concerning the tubular type of proteinuria in febrile diseases has not been published earlier On the other hand glomerular proteinuria occurred in 8 of the 10 patients studied sequentially and was uninfluenced by disappearance of fever in 4 of these patients

These findings imply that the tubular type of proteinuria was caused by fever per se and the increased urinary excretion of the low molecular weight proteins might be due to a transient impairment of the tubular reabsorptive capacity or to an increased plasma concentration of these proteins (6 19)

The glomerular type of proteinuria was probably not caused by fever per se since the urinary excretion of the high molecular weight proteins increased in 2 patients (nos 4 and 9) after the fever had subsided The glomerular proteinuria implies a glomerular injury possibly caused by an immune response to antigens derived from the infectious agents producing a diffuse or focal glomerulopathy of the same character as in complex glomerulonephritis (5) This assumption is supported by the fact that glomerular antigen–antibody complexes derived from various infectious agents have been demonstrated by immunofluorescent microscopy (3 18 20)

The duration of the glomerular type of proteinuria after disappearance of fever may largely depend upon the severity of the glomerular injury In most cases this injury probably is minor and the glomerular proteinuria will disappear within one or two weeks as demonstrated in most of our patients and in the series of Jensen and Henriksen (13) When the glomerular lesion is more serious the urinary excretion of the high molecular weight proteins may persist or even increase (Table III)

In conclusion our results demonstrate that febrile diseases are accompanied by a mixed glomerulo-tubular type of proteinuria The tubular proteinuria is caused by the fever per se and disappears rapidly once the fever subsides whereas the glomerular proteinuria if present may persist for one or two weeks

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Effect of Sodium Loading and Exercise on Renal Haemodynamics and Urinary Sodium Excretion in Young Patients with Essential Hypertension before and during Propranolol Treatment

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ABSTRACT The effects on renal plasma flow (RPF) and glomerular filtration rate (GFR) of an i v sodium load and exercise have been measured in 14 young patients with essential hypertension before and after 3–4 months treatment with propranolol as well as in 10 normotensive control subjects In the untreated hypertensive patients, RPF and GFR were significantly reduced during sodium loading and exercise After propranolol treatment, RPF and GFR were unaffected by sodium loading but decreased during exercise In the normotensive control group GFR and RPF were unchanged during sodium loading RPF decreased during exercise, whereas GFR was not significantly reduced RPF was significantly lower in the untreated hypertensive patients than in the normotensive control subjects during sodium loading and exercise Propranolol treatment induced a significant reduction of BP and heart rate RPF and GFR were not altered by propranolol treatment during sodium loading During exercise however RPF was significantly lower after treatment than before Urinary sodium excretion during sodium loading was significantly higher in the hypertensive patients than in the control subjects and decreased significantly during propranolol treatment The reason for the abnormal reduction in renal haemodynamics during sodium loading in patients with essential hypertension is not clarified but may possibly be related to functional or structural alterations in the renal vascular bed The lower RPF during exercise after treatment with propranolol is most likely caused by an inhibition of the cardiac output induced by β adrenergic blockade It is conceivable that the reduced urinary sodium excretion during propranolol treatment is attributable to the reduction of BP

In previous studies (1 2 4 6 7 9) patients with essential hypertension have been shown to excrete a sodium load faster than normotensive subjects The influence of an i v sodium load on renal haemodynamics is however disputed Some investigators (13 15) report an increase in glomerular filtration rate (GFR) and renal plasma flow (RPF) associated with sodium loading whereas others (6) have not found any changes It is well known that RPF is reduced during exercise (5)

According to earlier investigations β adrenergic blocking agents induce only small changes in renal haemodynamics in patients with essential hypertension at rest (11 13 17) Very little information is available about the effect of β adrenergic blockade on renal haemodynamics during sodium loading and exercise

Although the mechanism of exaggerated natriuresis after sodium loading in hypertensive patients has not been clarified it has been suggested (13) that a drop in BP during antihypertensive treatment induces a fall in the rate of sodium excretion during loading

The purpose of the present investigation was to measure RPF and GFR during sodium loading and exercise in a group of young patients with essential hypertension and in a normotensive group of control subjects to study the effect of propranolol on renal haemodynamics during sodium loading and exercise and to evaluate the effect of propranolol on the natriuretic response to sodium loading

SUBJECTS

Fourteen patients with essential hypertension 10 males and 4 females mean age 8 years (range 21–36) were examined

Table III *Comparison of RPF (ml/min) and GFR (ml/min) between untreated hypertensive patients and control subjects during sodium loading (period 2) exercise (period 4) and control periods (periods 1 3 and 5)*

	Untreated pats	Controls	*p* value
Period 1			
RPF	475	529	>0 05
GFR	113	123	>0 05
Period 2			
RPF	404	514	<0 01
GFR	95	114	>0 05
Period 3			
RPF	463	523	>0 05
GFR	108	118	>0 05
Period 4			
RPF	343	427	<0 05
GFR	90	108	>0 05
Period 5			
RPF	482	538	>0 05
GFR	110	115	>0 05

ences were found in RPF in the control periods GFR did not show any significant differences between the two groups in either of the periods

Comparison of RPF and GFR in hypertensive patients before and after propranolol treatment

Table IV shows the levels of RPF and GFR in 11 patients before and after treatment with propranolol RPF during exercise was significantly lower (*p*<0 05) after (277 ml/min) than before treatment (323 ml/min) No significant differences (*p*>0 05) were found in RPF in the other periods GFR was decreased (*p*<0 05) after propranolol treatment in period 3 but unchanged in the other periods

Effect of sodium loading and exercise on mean BP and heart rate

In the untreated hypertensive patients (Table V) mean BP increased slightly from 122 mmHg after one hour in the supine position to 128 mmHg after sodium loading (*p*<0 02) and to 129 mmHg after exercise (*p*<0 01) The heart rate increased from 69 to 111 beats/min (61%) after exercise (*p*<0 01) After sodium loading no changes were detected in the heart rate (73 beats/min *p*>0 05)

In the control subjects (Table V) changes in BP and heart rate varied in parallel with the untreated hypertensive patients Mean BP after one hour [in] the supine position was 92 mmHg and increased [to] 96 mmHg after sodium loading (*p*<0 01) and to [...] mmHg after exercise (*p*<0 01) The heart rate [in]creased from 64 to 109 beats/min (70%) after exe[r]cise (*p*<0 01) Sodium loading did not affect t[he] heart rate 68 beats/min (*p*>0 05)

After propranolol treatment (Table V) mean [BP] increased from 109 to 119 mmHg (*p*<0 01) aft[er] sodium loading and was 119 mmHg after exerci[se] (*p*<0 01) The heart rate increased from 56 to [...] beats/min (*p*<0 05) after the sodium load Aft[er] exercise the heart rate increased from 56 to [...] beats/min (39%) (*p*<0 01)

Comparison of mean BP and heart rate between untreated hypertensive patients and control subjects

As shown in Table VI, mean BP was significant[ly] lower (*p*<0 01) in the control subjects than in th[e] hypertensive patients after one hour in the supin[e] position during sodium loading and during exer[-]cise During the same periods heart rate was simila[r] in the two groups (*p*>0 05)

Comparison of mean BP and heart rate in hypertensive patients before and after treatment

Table VII shows significantly lower levels of mea[n] BP and heart rate during propranolol treatment aft[er]

Table IV *Comparison of RPF (ml/min) and GF[R] (ml/min) in 11 hypertensive patients before (B) an[d] after (A) propranolol treatment during sodium load[-] ing (period 2) exercise (period 4) and contr[ol] periods (periods 1 3 and 5)*

	B	A	*p* value
Period 1			
RPF	443	424	>0 05
GFR	108	104	>0 05
Period 2			
RPF	400	351	>0 05
GFR	93	90	>0 05
Period 3			
RPF	460	371	>0 05
GFR	104	92	<0 05
Period 4			
RPF	323	277	<0 05
GFR	85	78	>0 05
Period 5			
RPF	457	406	>0 05
GFR	104	100	>0 05

Table V *Mean BP (mmHg) and heart rate (beats/min) after one hour in the supine position sodium loading and exercise in 14 untreated hypertensive patients 10 controls and 11 propranolol treated patients*

Subj no	After 1 h in the supine position		After sodium loading		After exercise	
	Mean BP	Heart rate	Mean BP	Heart rate	Mean BP	Heart rate
Untreated patients						
1	123	78	123	82	127	120
2	120	64	135	60	123	100
3	120	68	137	80	140	124
4	152	72	153	76	150	100
5	117	72	115	74	127	116
6	105	79	110	78	107	140
7	117	84	130	96	132	128
8	120	56	118	50	120	80
9	112	60	128	84	128	104
10	123	48	125	60	128	68
11	137	80	143	70	138	140
12	118	70	128	78	133	106
13	130	68	137	70	132	112
14	117	72	112	70	115	110
Mean	122	69	128	73	129	111
S D	11	10	12	12	11	20
Controls						
1	93	68	98	68	97	120
2	93	54	98	68	95	88
3	92	66	90	68	93	90
4	92	68	97	90	102	108
5	85	70	92	74	98	150
6	103	66	107	52	105	104
7	88	56	95	62	95	102
8	93	66	95	65	98	98
9	92	64	93	68	95	120
10	87	64	92	68	95	108
Mean	92	64	96	68	97	109
S D	5	5	5	10	4	18
Treated patients						
1	95	60	123	70	115	86
2	118	60	123	62	122	66
3	112	48	117	44	117	60
4	125	56	153	60	132	68
5	110	58	107	58	117	88
6	98	74	110	78	103	98
7	117	50	130	52	127	92
8	103	50	105	52	107	62
9	98	54	112	64	120	88
10	102	42	107	46	117	60
11	122	62	127	70	127	94
Mean	109	56	119	60	119	78
S D	11	9	14	11	9	15

one hour in the supine position ($p<0\ 01$) during sodium loading ($p<0\ 02$) and during exercise ($p<0\ 01$)

Effect of sodium loading on urinary sodium excretion

Table VIII shows urinary sodium excretion before during and after sodium loading in the untreated hypertensive patients controls and propranolol treated patients The pretreatment values increased from 7 37 mEq in period 1 to 17 56 mEq in period 2 ($p<0\ 01$) and further to 27 78 mEq ($p<0\ 01$) during period 3 In the control subjects urinary sodium excretion increased from 4 69 mEq in period 1 to 9 81 mEq in period 2 ($p<0\ 01$) and further to 24 01 mEq during period 3 ($p<0\ 01$) The

Table VI *Comparison of mean BP (mmHg) and heart rate (beats/min) between untreated hyperten sive patients and control subjects after one hour in the supine position sodium loading and exercise*

	Untreated pats	Controls	*p* value
After 1 h in the supine position			
Mean BP	122	92	<0 01
Heart rate	69	64	>0 05
After sodium loading			
Mean BP	128	96	<0 01
Heart rate	73	68	>0 05
After exercise			
Mean BP	129	97	<0 01
Heart rate	111	109	>0 05

propranolol treated patients showed increases from 7 10 mEq to 14 08 during period 2 ($p<0\ 01$) and *further to 23 06 during period 3 ($p<0\ 01$)*

Comparison of urinary sodium excretion in the untreated hypertensive patients and control subjects

Urinary sodium excretion was significantly higher during sodium loading in the untreated hypertensive patients (17 56 mEq) than in the normotensive con trol subjects (9 81) ($p<0\ 02$) The values before nd after loading did not differ significantly ~0 05)

Comparison of urinary sodium excretion in hypertensive patients before and after treatment

During sodium loading the urinary sodium excre tion (14 08 mEq) in the propranolol treated patients was significantly lower ($p<0\ 05$) than before treat ment (18 90 mEq) No significant effect on sodium excretion was found in periods 1 and 3 ($p>0\ 05$)

Relationship between changes in sodium excretion and changes in BP during sodium loading

Changes in sodium excretion during propranolol treatment in period 2 were not correlated to changes in mean BP either after one hour in the supine position (rho=−0 102 $n=11$ $p>0\ 05$) or after sodium loading (rho=0 602 $n=11$ $p>0\ 05$)

DISCUSSION

The present results show that i v sodium loadi induced a drop in RPF and GFR in patien with essential hypertension This reduction w abolished during treatment with propranolol ar was not found in the normotensive control subject

Cottier et al (6) did not find any changes in inul and para aminohippurate clearance and mean BP patients with essential hypertension during eith i v loading with 500 ml 2 5% sodium chloride the following control periods This is in contrast the results of Krauss et al (13) and Schalekamp al (19) who found a slight increase in ^{125}I hippura clearance after i v infusion of 300 ml 5% NaC *In the two latter studies however renal ha*modynamics were not measured during infu sion The increase in renal blood flow was as sociated with elevated cardiac output and de creased total peripheral resistance and it was sug gested that the changes in renal haemodynamic could be attributed to changes in systemic haemodynamics Although Lowenstein et al (15 measured an increase in RPF and GFR during load ing with 1 000 ml 2 5% NaCl they could not dem onstrate any systemic alterations They did how ever find a decrease in renal vascular resistance during sodium loading and their results suggested unlike those of Krauss et al (13) that the changes in the renal vascular bed were responsible for the alterations in RPF and GFR during loading with sodium

Although the changes in renal haemodynamics and mean BP were small in the present study the

Table VII *Comparison of mean BP (mmHg) and heart rate (beats/min) before (B) and after (A) propranolol treatment after one hour in the supine position sodium loading and exercise*

	B	A	*p*-value
After 1 h in the supine position			
Mean BP	122	109	<0 01
Heart rate	69	56	<0 01
After sodium loading			
Mean BP	129	119	<0 02
Heart rate	74	61	<0 07
After exercise			
Mean BP	129	119	<0 01
Heart rate	111	78	<0 01

Table VIII *Urinary sodium excretion (mEq/40 min) before (period 1) during (period 2) and after sodium loading (period 3) in 14 untreated hypertensive patients 10 controls and 11 propranolol treated patients*

Subj no	Period 1	Period 2	Period 3
Untreated patients			
1	5 66	10 40	8 18
2	11 16	29 73	28 22
3	18 21	23 84	30 03
4	10 10	18 17	34 84
5	12 04	20 24	55 62
6	3 26	7 37	17 09
7	13 01	30 05	36 90
8	3 16	19 42	22 84
9	1 92	18 44	44.54
10	9 02	19 75	25 25
11	3 37	10 47	14 45
12	4 34	15.34	15 35
13	4 44	11 64	16 52
14	3 50	10 95	11 14
Mean	7.37	17 56	27 78
S D	4 90	7 07	13 60
Controls			
1	5 04	6 80	32 71
2	5 85	13 35	22 67
3	4 35	8 11	8 61
4	4 66	12 90	43 08
5	6 38	5 99	8 01
6	3 44	10 21	12 30
7	1 20	1 17	4 58
8	8 91	11 75	51 45
9	4 01	23 93	51 46
10	3 10	3 89	5 26
Mean	4 69	9 81	24 01
S D	2 08	6 34	19 17
Treated patients			
1	4 40	14 50	17 18
2	4 92	9 68	11 27
3	7 89	11 34	19 34
4	11 92	12 92	20 89
5	6 35	20 03	38 85
6	1 20	7 00	11 30
7	10 56	31 92	31 88
8	13 48	12 90	28 87
9	3 05	9 61	39 23
10	6 26	17 35	26 10
11	8 10	7 66	8 70
Mean	7 10	14 08	23 06
S D	3 77	7 11	10 81

elevated BP associated with a decreased RPF suggests an increase in the renal vascular resistance during sodium loading This disagreement with the results of the three investigations mentioned above is difficult to explain but may be related to differences in the responsiveness of the renal vascular bed or to differences in the amount of sodium loaded The present patients were younger than those in the above studies and thus may have another degree of functional or structural lesion in the renal arterioles Furthermore a larger sodium load was given during a shorter period It is conceivable that differences in RPF and GFR during sodium loading are attributable to differences in both the renal vascular lesions and the sodium load However the mechanism responsible for the normal response of RPF and GFR to sodium load during treatment is obscure but might possibly be related to a reduction of BP

According to the present study exercise reduced RPF and GFR in the normotensive subjects as well as in the hypertensive patients both before and after treatment Mean BP and heart rate increased as expected in both The changes in RPF and systemic haemodynamics were thus the same as those seen in exercise experiments without preceding sodium loading (5)

Patients with moderate and severe essential hypertension have decreased RPF (8 14) whereas it is often within normal ranges in patients with mild hypertensive disease (10 18) This finding is in agreement with the present results which did not show any significant differences in RPF between hypertensive and control subjects during the control periods During sodium loading and exercise RPF was clearly lower in the hypertensive patients than in the control subjects It was thus confirmed that an abnormality concerning regulation of renal blood flow exists in patients with essential hypertension However this abnormality is not always demonstrated during basal circumstances but may be revealed during sodium loading and exercise

Beta adrenergic blocking agents induce only small changes in renal haemodynamics at rest During propranolol treatment Ibsen and Sederberg Olsen (11) noticed a slight decrease in ^{51}Cr EDTA clearance in patients with arterial hypertension and Krauss et al (13) found a small reduction in ^{125}I hippuran clearance in patients with essential hypertension In a previous study (17) we found no changes in RPF and GFR in patients with essential hypertension during alprenolol treatment a finding that agrees with the present results which show that renal haemodynamics were almost unaffected

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Decrease in α-Lipoprotein Cholesterol in Men after Cessation of Exposure to Chlorinated Hydrocarbon Pesticides

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ABSTRACT Eight men who in 1970 had been exposed to chlorinated pesticides and for whom the exposure had ceased in 1976 showed a significant fall in α lipoprotein (HDL) cholesterol between these years The cholesterol concentration of the other lipoprotein classes showed no significant trend The data support our previous suggestion that exposure to chlorinated pesticides, mainly lindane may raise the α lipoprotein levels

We previously reported that of 22 men exposed in their occupation to a mixture of chlorinated pesticides 40% had hyper-α lipoproteinaemia (4) We have now 6 years later had an opportunity to reexamine the α lipoprotein cholesterol levels in 8 subjects exposed and studied in 1970 when the exposure had disappeared

SUBJECTS

Eight men who were occupationally exposed to lindane were examined They sprayed a solution containing 4% lindane 0 1% pyrethrum and 2 5% malathion A few had been exposed to DDT in the period 1955–65 and three of them intermittingly also to pentachlorophenol Sometimes they diluted concentrated stock solutions to prepare the mixture mentioned above They were supposed to wear protective clothing and masks but this recommendation was not always followed Lindane concentration in the air was not measured The mean lindane level in this group of spray-men was 18 4 ng/ml (range 0–87) as reported previously (5) The men did not have a fixed place of work Observing them during a working shift one of us (BKH) found that both inhalation and skin contact were possible The frequency of exposure varied from daily to once weekly The duration of employment varied between one and 25 years Lindane exposure was discontinued in 1974

METHODS

Blood was drawn by venipuncture in the morning after fasting overnight under the same conditions as in the initial study (4) Serum was recovered after 1–2 hours at room temperature mixed with 5% EDTA to a final concentration of 0 05% and centrifuged in a Spinco preparative ultracentrifuge in a 40 3 rotor at $d=1\ 006$ as described earlier (2) The top fraction very low density lipoproteins (VLDL) was recovered by tube slicing and low density lipoproteins (LDL) were precipitated in the infranatant with $MgCl_2$-heparin according to Burstein and Samaille (1) leaving high density lipoproteins (HDL) in solution Cholesterol and triglycerides were determined in total serum VLDL the LDL+HDL fraction and the HDL fraction (2) The values obtained for HDL cholesterol by the present method using only one ultracentrifugal spin are identical (2) to those obtained by the method used in the initial study (4) which involved two ultracentrifugal spins

RESULTS AND DISCUSSION

In seven of the eight subjects the HDL cholesterol was lower in 1976 than in 1970 (Table I) the average individual decrease being $12\ 5\pm2\ 1$ (S E M) mg/100 ml ($p<0\ 001$ paired t test) The corresponding figures for LDL and VLDL cholesterol were 15 ± 8 ($p>0\ 05$) and $-2\ 5\pm1$ ($p>0\ 05$) Thus the only significant change in lipoprotein concentrations since 1970 was the fall in HDL cholesterol Since HDL cholesterol does not decrease with age (3) it is tempting to ascribe the fall to the cessation of occupational exposure to chlorinated pesticides This seems particularly likely as we previously found that men exposed to such pesticides had a high frequency of hyper-α lipoproteinaemia (4) Also in animal experiments (rabbits and rats) we

T ible I Serum HDL cholesterel (mg/100 n l) an l degree f exp sure t chl ri ated pesti ides

S bj n in previous p bl cat n (4)	Degree of expos re		Ye rs of expo sure	HDL ch lester l	
	1970	1976		1970	1976
8	+++	0	5	72	57
17	+++	0	35	76	6?
10	+++	0	6	148	127
13	+++	0	5	56	41
4	+++	0	1	64	55
5	+++	0	4	86	70
12	+++	0	6	98	99
-	+	0	13	54	44

found that lind ine feed ng at 100 ppm level r ised HD cholesterol (6) It is known th it the domestic b ickgro ind of lind ine in the pl sm i of Swedes is below the detect on l m t of 0 2 ng/ml or < 1 0 ng/ml (6 7 8)

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The Significance of AV Block I in Asymptomatic Young Men

Variability some Anthropometric Data Orthostatic Test Reaction and Physical Work Capacity

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ABSTRACT Twenty nine young men with AV block I (P R interval >0 22 sec), without any history of heart disease, have been compared with 112 randomly selected healthy men of the same age In 24 subjects who were free from concomitant ECG changes the P R interval was normalized during orthostatic test and/or physical exercise This group did not differ from the controls regarding anthropometric data and physical work capacity except for a higher heart rate at rest, thus suggesting that in these subjects an occurrence of AV block I has no pathological significance However, it cannot be excluded that the block is a sequela, for instance to myocarditis Three subjects with AV block I and inverted T waves in the precordial leads and two subjects with unchanged P R intervals to increased sympathetic tone had on average a smaller body size a higher HR and systolic BP in recumbent position and smaller blood volumes

The P R interval in human surface ECG reflecting the atrioventricular conduction time is normally less than 0 20 sec at rest (12) and an interval exceeding 0 21 sec is generally considered as an AV block I (5 16) and thereby as an impaired atrioventricular conduction of possible pathological significance However cases with a long P R interval as a single symptom who seem to be quite healthy and have no history of cardiac disease are rather common The incidence of AV block I in young men has been found to be 0 45–1 1% (3 10 14 15) Very few of these young men had any history of heart disease or concomitant ECG changes Most of them had a decrease in and often a normalization of their P R interval during an orthostatic or exercise test (3 10 14) Thus a delay in atrioventricular conduction reflected by a prolonged P R interval appears to be a benign condition and to a great extent might be an extreme variation within the physiological range

The aim of our study has been to determine the significance of AV block I in young men examined before military service particularly regarding physical work capacity and some anthropometric data We have taken the opportunity of obtaining a control group from the same enlistment centre where practically all men 18–19 years of age from widespread parts of Sweden are examined

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SUBJECTS AND METHODS

Of a large number of conscripts aged about 18 years examined at the Enlistment Centre Solna Sweden 29 subjects with AV block I were selected for further examination at Karolinska Hospital on the following day Since the examination capacity of the hospital was limited and subjects with some other ECG disorders were given priority it was not possible to examine all subjects found to have AV block I so prevalence data cannot be given AV block I was defined as a P R interval of more than 0 22 sec at rest Subjects with a known history of heart disease were excluded Comparisons are made with 112 controls reported elsewhere (1)

Heart volume (HV) was estimated in supine position (11) Blood volume (BV) was calculated from a peripheral haematocrit and single estimation of total Hb determined by the alveolar CO method (8) An orthostatic test was performed including heart rate (HR) blood pressure (BP) and ECG after standardized standing for 8 min The exercise test was performed in the sitting position on an electrodynamically braked cycle ergometer (EM 370-I Siemens Elema) with stepwise increasing loads every 6t

Table I *Some anthropometric and other data in 112 controls in 24 subjects with AV block I normalized by orthostatic test or exercise (group A) and in 2 subjects with AV block I persisting during orthostatic test and exercise and 3 subjects with pathological T wave changes (group B)*

	Controls (C)		Group A		Group B		Significance of difference ($p <$) between groups		
	Mean	*S D*	*Mean*	*S D*	*Mean*	*S D*	*A & C*	*B & C*	*A & B*
Height (m)	1 80	0 065	1 80	0 051	1 74	0 043	(0 90)	0 05	0 05
Weight (kg)	68 3	10 0	67 8	9 0	63 2	4 1	(0 90)	(0 30)	(0 30)
HR at rest (beats/min)	66	11	72	15	83	18	0 025	0 005	0 005
HR in orthostatic test (beats/min)	85	13	89	15	92	15	(0 20)	(0 30)	(0 30)
Systolic BP recumbent (mmHg)	125	12	130	13	138	8	(0 10)	0 02	(0 70)
Diastolic BP recumbent (mmHg)	74	8	77	5	79	6	(0 10)	(0 20)	(0.50)
Systolic BP standing (mmHg)	125	10	127	14	131	7	(0 50)	(0 20)	(0 60)
Diastolic BP standing (mmHg)	81	8	80	6	82	8	(0 60)	(0 90)	(0 60)
Blood volume (l)	5 6	0 7	5 7	0 8	4 8	0 7	(0 70)	0 02	0 05
HV (ml)	771	118	781	129	729	112	(0 80)	(0.50)	(0 50)
W_{170} (W)	1 033	219	1 041	238	950	292	(0 90)	(0 50)	(0.50)

min up to a HR of about 170 beats/min (19) The load giving a HR of 170 beats/min (W_{170}) was used as a measure of the physical work capacity ECG was recorded with an ink jet electrocardiograph (Mingograph 81 Siemens Elema) using CR leads at rest and chest head leads during exercise (9) BP was measured with a calibrated cuff on the right upper arm by auscultation The observed phase 5 value was rounded off to the nearest level of 5 mmHg ifferences between group means were tested for signifi ce using the t-distribution

RESULTS

The physical examination of the 29 subjects revealed no sign of cardiac disease Mean P R interval at the enlistment centre was 0 26 sec (range 0 23–0 40)

At the hospital examination the P R interval was normal in 8 subjects and in two cases it was further prolonged In three cases there were flat or inverted T waves in precordial leads mainly localized in CR_5 and these changes were accentuated by the orthostatic test and partly or totally normalized during the exercise test These ECG changes were regarded as sequelae to a myocarditis In the remaining 26 subjects the AV block I was the only ECG change In 25 cases the block disappeared during the orthostatic test and in 27 subjects the P R interval was normalized during exercise In the two subjects with unchanged P R interval during orthostatic test as well as during exercise the AV block I was regarded as pathological and these two subjects together with the three subjects with pathological T wave changes formed a separate AV block I group (group B) Their mean P R inter val was 0 27 sec (range 0 24–0 37)

The 24 subjects without concomitant ECG changes and with a normalization of the P R inter val in response to increased sympathetic tone formed group A Their mean P R interval was 0 2! sec (range 0 23–0 40) Group A did not differ from the controls in any measured variable except for HR at rest which was on an average 6 beats/mir higher among the subjects with AV block I (Table I) The relation between W_{170} and total Hb and HV for group A did not differ from that in the controls

The mean height in group B was 65 mm less than in group A BV 0 9 l less and HR at rest was 1 beats/min higher Compared with the controls group B had higher systolic BP in recumbent posi tion (Table I)

DISCUSSION

AV block I is rather common in all age groups The problem lies in separating the functional from the organic blocks In the lower age groups rather few of the subjects with a prolonged P R interva have a history of heart disease and/or findings a physical examination Some have concomitar

ECG changes In the majority the AV block I is an isolated finding (3 10 14 15) First degree AV block occurring in the absence of bundle branch block as in the present subjects generally reflects a delay proximal to the bundle of His probably in the AV node (7) and is thereby within the reach of physiological stimuli (6 20 21) It is therefore of advantage to extend the examination with a stress test such as an orthostatic test or exercise in order to decrease vagal tone and/or increase sympathetic tone which normally initiates a decrease in P R interval (4) In this age group most of the prolonged P R intervals without broad QRS complexes will decrease and even become normal in response to a stress (3 10 14 18) The AV block I at rest in these subjects must be regarded as functional and the prolonged P R interval might be an extreme value within the physiological range In this study AV block I was regarded as functional in 24 out of 29 cases The functional cases did not differ from the controls regarding height weight BP BV HV or physical work capacity which supports the view that they should be regarded as normals

A possible explanation for the prolonged P R interval might be an increased basal vagal tone since the vagal tone is known to increase the delay of the impulse in the AV node Subjects with a high physical work capacity are known to have a higher basal vagal tone and an increased incidence of AV block I (12) However group A did not differ significantly from the controls regarding physical work capacity HR at rest was even higher than in the controls which is inconsistent with a higher basal vagal tone Since the basal sympathetic tone at rest in recumbent position is slight (4 17) a reduced sympathetic tone cannot explain the prolongation of the P R interval and such a mechanism is also contradicted by the higher HR at rest found in the groups with AV block I compared with the controls

However it cannot be excluded that the prolongation of the P R interval is due to an increased sensitivity to vagal tone in the AV node resulting from local damage by for instance a myocarditis In this study the three subjects with other signs of myocardial damage the T wave changes might be an example of this mechanism provided their AV block I and T wave changes have the same genesis since their P R interval reacted physiologically to an orthostatic and exercise stress with a shortening of the interval to normal values

The five subjects whose AV block I was regarded as organic (group B) differed from the controls regarding anthropometric data in a way similar to that of a group of 64 subjects with pathological T wave changes examined parallel to the subjects with AV block I at the same enlistment centre (2) suggesting that group B actually belonged rather to a group with ECG signs of organic heart disease than to a functional ECG change group

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Amoeboid Movement Configuration in Tumour Cells of Bone Marrow Smears from Patients with Leukaemia

Incidence and Significance

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ABSTRACT The incidence of amoeboid movement configuration (AMC), a cell shape suggestive of cell locomotion at the moment of fixation, has been studied in the tumour cells of bone marrow smears from leukaemia patients at the time of diagnosis The groups of patients with CML ($n=8$), ALL ($n=5$) and CLL ($n=9$) were small, and the incidences of AMC were close to those found in the corresponding cell lines from healthy probands In 39 patients with AML, the incidence of AMC was higher than in the other cell lines investigated A positive skew distribution of AMC values and a positive significant correlation between incidence of AMC were found at the time of diagnosis and subsequent survival of the patients with AML, in spite of differences in treatment It is suggested that this positive correlation may be due to an immune reaction of the patients against their tumour cells

Cells in locomotion have a polarized elongated or triangular shape which they retain in fixed specimens In a previous study we noted that 40–50% of the tumour cells in the bone marrow smears from two patients with acute myeloblastic leukaemia (AML) and one patient with a lymphosarcoma displayed the characteristic *amoeboid movement configuration* (AMC) suggestive of active locomotion at the moment of fixation (7) Time lapse filming of coverslip preparations of the bone marrow of two of the patients mentioned verified that the majority of tumour cells with AMC actually moved Two patients with less conspicuous incidences of AMC one with AML and one with multiple myeloma were also described (7) This observation suggested to us that the incidences of tumour cells with AMC do not represent an all or none phenomenon but form some kind of continuous distribution

The aim of the present study was to describe the incidence of AMC in the tumour cells of the bone marrow smears from leukaemia patients at the time of diagnosis prior to any treatment Contrary to our expectations there was a strong positive correlation between incidence of AMC and length of survival in patients with AML or acute myelomonocytic leukaemia (AMML) This covariation was further examined in two other populations of non treated and treated patients with AML

MATERIAL AND METHODS

The incidence of AMC was determined in bone marrow smears from the patients with leukaemia admitted to the Department of Internal Medicine University Hospital Lund in 1971 (Tables I and II) The leukaemic patients were grouped as AML or AMML chronic myeloid leukaemia (CML) acute lymphoblastic leukaemia (ALL) and chronic lymphocytic leukaemia (CLL)

The criteria for inclusion in the study were that the disease was diagnosed just prior to or during the first contact with the department that the patient had not received cytostatic treatment prior to the sternal aspirate examined that the patient survived at least one week after the diagnostic puncture and that the smears were considered representative for the bone marrow as regards cellularity and quality Thus 8 patients (4 AML 1 CML 3 CLL) were excluded due to hypocellularity Four patients (2 AML 1 CML 1 CLL) succumbed during the first 4 days in hospital due to pneumonia Two patients with rare variants of leukaemia were also excluded one with promyelocytic leukaemia and one with monocytic leukaemia of histiocytic type Only patients with typical CLL were studied not patients with generalized lymphosarcoma

The finding of a significant positive correlation between incidence of AMC and survival of patients with AML AMML warranted an extension of this study Thus 19 patients with AML diagnosed in 1968–72 were examined concerning AMC in the pretreatment smears from the

Table I *Pat ents with acute myeloblast c (AML n=11) or acute myelomonocyt c (AMML n=2) leukaemia d agnosed in 1971 treatment incidence of amoebo d movement configurat on (AMC) (% of tumour cells in the bone marrow smear prior to treatment) and surv val after d agnost c puncture*

VAMP cycl c treatment with vin ristine methylam nopterine 6-mercaptopurine and predn solone Q_1–Q_3= interquart le range

Pat age (y)	Sex	D ag nos s	AMC	Sur vival (weeks)	Cytostatic treatment
68	♂	AML	?0	4	6-mercaptopurine
83	♀	AMML	14 6	18	None
60	♂	AML	9 4	3	VAMP
78	♀	AML	?3	1	None
73	♀	AML	35 6	25	VAMP
48	♂	AML	23 5	79	VAMP
?9	♀	AML	15 9	4	VAMP
44	♂	AML	8 5	7	V nblastine
65	♂	AML	18 5	15	None
15	♂	AML	13 3	14	VAMP
?0	♀	AML	6 9	5?	VAMP
62	♀	AML	6 0	1?	VAMP
4?	♀	AMML	16 7	36	None
Med an			14 6	14	
Q_1–Q_3			7 3–?1 6	4–30 5	

bone marrow After d agnos s these pat ents were treat ed with cycl vincrist ne-methylam nopterine-6-mercaptopurine-prednisol ne (VAMP) (Table III) The correla between AMC and surv val was also stud ed n 9 el pat ents w th AML d agno ed in 1968–7? and only symptomat ally w th blood transfus ons and prednisol ne (Table III)

AMC was def ned as a rat o long cell ax s/short cell ax s ≥? (F g 1) 1 000 tumour cells were counted in each proband Control counts showed that the present counts of AMC performed by Sjögren corresponded to the counts of AMC in the previous study (7) with n the l m ts of the methodolog al error Presumably normal bone marrow lymphocytes 250 cells donor (controls versus CLL lym phocytes Table II) were obta ned from 9 female and 6 male probands without perce vable haematological d or der and with cl n cal d agnoses such as spondyl sthesis cervical d sc prolap e ad positas p ych atr d sorders Their age was 19–8? years (mean 50) Some pertinent haematological data were ESR 23 mm (two women w th ESR ?1 and 23 mm respect ely were more than 60 years old) Hb 1? 1 16 6 g/100 ml RBC 3 8 5 1 m ll /µl WBC 2 ?00–8 300 µl

Non parametric statist s were used tab ed and cal u lated accord ng to S egel (10) The Spearman rank correla t on test was corrected for t es the Mann Wh tney test was not The two-tailed d stributions of the tests were used Levels of s gn ficance $p>0.05$=not sign f cant $0.05>p>0.01$=almost s gn f ant $0.01>p>0.001$=s gn f icant $0.001>p$=h ghly signif ant

RESULTS

Acute myel blastic and myel m nocyt c leukaem a It is evident from Table I that th incidence of AMC in tumour cells from the bone marrow smears of 13 patients with AML AMML was considerably h gher than in myeloblasts f om healthy donors (4 4%) as reported previously (7) The correlat on between incidence of AMC and weeks of survival was positive (R_s=−0 502) and sign ficant ($0.01>p$)

Chron c myel d leukaem a The incidence of AMC in tumour cells from patients with CML (Table II) was sign ficantly lower ($0.01>p$) than th from patients with AML (Table I) The incidence in CML patients (Table II) was on the same

Table II *Pat ents with chr n c myel id (CML) acute l mphoblast c (ALL) and chr n c lymphocyt c leukaem a (CLL) incidence of amoeboid m ement conf gurat n (AMC) (% of t mour cel in the bone marr smear pri r t treatmen c d surv val after d agnost c puncture*

Pat age (y)	Sex	AMC	Survival (weeks)
CML			
5?	♂	4 1	?5
59	♂	?0	39
4	♂	8 8	>198 alive
56	♀	4 8	11
60	♀	4 4	158
30	♀	4 0	>231 a e
4?	♀	5?	179
62	♂	7 6	>?14 al e
Median		4 6	169
ALL			
18	♂	14 7	62
5	♂	3 7	6
?	♂	5 1	>193 a e
17	♂	8 6	?3
4	♀	9 8	>?3 a e
Median		8 6	6?
CLL			
?0	♂	5 9	>?3 a e
5?	♂	16 7	>195 a e
57	♀	4 5	133
3	♂	1 7	108
6	♀	6 4	80
60	♂	4 0	>198 a e
65	♂	9 ?	191 al e
83	♂	6 6	55
8	♂	4 7	13
Median		5 9	108

absolute level as in myeloblasts from healthy donors as reported earlier (7) The patients with CML were too few to allow a statistical analysis of the correlation between AMC and survival

Acute lymphoblastic leukaemia Only 5 patients were available for analysis (Table II) The incidence of AMC varied on the same absolute level as in bone marrow lymphocytes from healthy donors (see below)

Chronic lymphocytic leukaemia The incidence

Table III *Patients with acute myeloblastic leukaemia (AML) treated in 1968–1972 with either cyclic vincristine methylaminopterine 6 mercaptopurine and prednisolone (VAMP) or blood transfusions and prednisolone incidence of amoeboid movement configuration (AMC) (% of tumour cells in the bone marrow smear prior to treatment) and survival after diagnostic puncture*

Q_1–Q_3=interquartile range

Pat age (y)	Sex	AMC	Survival (weeks)
VAMP			
25	♂	7 2	4
67	♂	1 7	3
33	♀	4 4	14
29	♂	7 5	25
62	♀	6 0	12
81	♂	3 4	4
50	♀	10 7	6
74	♀	4 9	2
15	♂	13 3	14
70	♀	26 9	52
29	♀	15 9	4
48	♂	23 5	79
73	♀	35 6	25
40	♂	36 2	36
51	♂	20 9	29
60	♂	9 4	3
47	♂	27 3	>149 alive
65	♀	34 9	5
79	♂	16 8	107
Median		13 3	14
Q_1–Q_3		6 0–26 9	4–36
Blood transfusions and prednisolone			
69	♂	47 4	12
57	♂	4 0	2
81	♀	6 9	3
8	♀	2 3	1
65	♂	18 5	15
60	♀	2 4	6
85	♀	7 3	3
76	♂	2 7	3
77	♀	2 0	1
Median		4 0	3



Fig 1 Amoeboid movement configuration (AMC) in tumour cells from a bone marrow smear of a patient with *acute myeloblastic leukaemia* Presumed direction of locomotion at the moment of fixation is marked by arrows In the cell counts the AMC was defined as a quotient long cell axis/short cell axis ≥2 (cell *a* =2 4) This strict definition excluded some cells with the hand-mirror shape typical of locomotion (cell *b* =1 7) May-Grunwald-Giemsa Basic magnification ×204

of AMC in tumour cells from patients with CLL was low (Table II) and on the same absolute level as in the lymphocytes of bone marrow smears from 15 healthy donors (median 6 8% Q_1–Q_3 4 8–9 6%) The slight difference between normal lymphocytes and CLL lymphocytes was not significant

The correlation between AMC and survival in AML It seemed desirable to reexamine the correlation between incidence of AMC in tumour cells and length of survival in patients with AML AMML (Table I) in other patient materials treated according to uniform therapy lines

In 19 patients with VAMP treated AML in 1968–72 (Table III) the correlation between incidence of AMC in tumour cells and length of patient survival was still positive (R =+0 654) and significant (0 01>p)

Only symptomatic treatment with blood transfusions and prednisolone was given to 9 elderly patients with AML in 1968–72 In this material the correlation between AMC of tumour cells and length of patient survival was positive (R_s=+0 758) and almost significant (0 05>p)

From the observations in Tables I and III it seems reasonable to conclude that there was a significant positive correlation between the incidence of AMC and the length of survival in pa with AML and AML AMML (Figs ?

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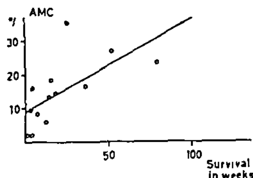


Fig 2 Correlation between incidence of AMC at the time of diagnosis and weeks of subsequent survival in 13 patients with AML AMML (Table I) $y=0.272x+9.22$ $R_s=+0.802$ $0.01>p$

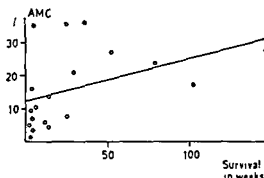


Fig 3 Correlation between incidence of AMC at the time of diagnosis and weeks of subsequent survival in 19 patients with VAMP treated AML (Table III) $y=0.125x+12.41$ $R_s=+0.655$ $0.01>p$

DISCUSSION

The present study provides evidence that the incidence of AMC in tumour cells from bone marrow smears of patients with leukaemia forms a continuous distribution The findings in patients with CML ALL and CLL do not warrant further discussion the populations were too small and the incidences obtained were too close to those of the corresponding cell lines from healthy donors as reported in a previous study (7) In 39 patients with AML and 2 with AMML (Tables I and III) the AMC values had a positive skew distribution as in Fig 4

The main observation in the present study was that the incidence of AMC is often high in the tumour cells from patients with AML AMML and considerably higher than in the corresponding cell lines from healthy donors as reported previously (7) The incidence of AMC at the time of diagnosis had a positive correlation to the survival of the patients in spite of differences in subsequent treatment

The above mentioned positive correlation is thought to reflect a real difference between patients with low and high incidences of AMC in the smear morphology of their tumour cells the patient groups studied were small and patients with AML are liable to succumb prior to the final stage of the basic disease due to infections and therapeutic complications The statistical tests operate conservatively in the present sample sizes i e the null hypothesis is favoured It is further favoured by premature deaths due to infections and complications of therapy the impact of which are heavy on the statistical significance of basic differences in small samples As a consequence only gross basic differences are supported by positive statistical evidence in samples of the present size

It is evident from Figs 2 and 4 that the exclusion of patients who died during the first week after diagnosis is not a selection in favour of the observed positive correlation between incidence of AMC and weeks of survival The presence of correlation or non correlation can as easily be observed after the first week but deaths due to non treatable infections during the first week would contaminate the statistical analysis of the following period These

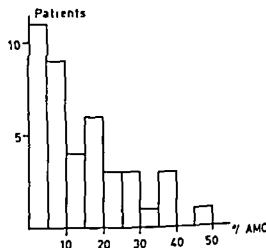


Fig 4 Distribution of AMC values in the tumour cells of the bone marrow smears of 39 patients with AML and 2 with AMML 1000 tumour cells counted per patient from Tables I and III This distribution is distinguished by a positive skewness

considerations warrant the exclusion from this study of non-evaluable patients who died during the first week after diagnosis

The cytobiological significance of AMC is at present not entirely clear Analysis of the information available on cell surface and cell locomotion may however provide some interesting clues Experiments by means of heat polarized anucleate cytoplasmic fragments of polymorphonuclear leucocytes (PMNs) appear to indicate that PMN locomotion and PMN chemotaxis are independent of cell nucleus as reported by Keller and Bessis (6) Experiments by means of antitubulins appear to indicate that cytoplasmic microtubules have a role in the direction finding during PMN chemotaxis but not in the random locomotion of PMNs as reported by Bandmann et al (2)

The considerations above seem to exclude nucleus and microtubules from the discussion on AMC and focus the interest on the cell membrane which is known to trigger the cell into locomotion or modify locomotion by adherence in many cell systems Phytohaemagglutinin stimulation of cultured lymphocytes from peripheral blood was reported to induce uropod formation—presumably equivalent to the present AMC—in 20% of the cells (4) The chemotactic response of PMNs is reported to be mediated by the interaction of chemotactic factors with the cell surface (5 6) which is thought to trigger and direct PMN locomotion Complementary receptors fixed on a marcrophage (11 12 13) on a nylon fibre (9) or on a glass surface (14) have been reported to induce the configuration of locomotion in normal lymphocytes At the same time the adherence of the lymphocyte tail to the substrate fixed complementary receptors is reported to *slow down or completely inhibit cell translocation* for periods of varying length Tumour cell invasion seems to presuppose active locomotion without adequate control mechanisms (1 3 8)

The above mentioned observations on AMC in cells from healthy donors could be consistent with the hypotheses that AMC in tumour cells of AML patients reflects either a mechanism in tumour cell invasion or a membrane activation by extrinsic or intrinsic factors The positive correlation observed in the present study between incidence of AMC and survival of the patients suggests that the hypothesis of AMC as a link in tumour cell invasion in AML should be rejected at least temporarily in favour of the hypothesis of membrane activation Since analogous membrane activations with formation of AMC in vitro often provide steps in immune reactions we find it reasonable to assume that AMC in AML may be due to an immune response of the patient against his tumour cells This idea is in agreement with the positive correlation observed between incidence of AMC and survival in patients with AML

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Prolonged Monosymptomatic Fever due to Yersinia Enterocolitica

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ABSTRACT A previously healthy 14 year-old girl showed monosymptomatic hectic fever for over 3 weeks with negative clinical findings Extensive laboratory investigation revealed only elevated ESR and a high titer of agglutinating antibodies against Yersinia enterocolitica serotype 0–3 These abnormalities disappeared upon tetracycline therapy, after which she remained in excellent health This unusual manifestation of yersiniosis which was of unknown source, demonstrates that Yersinia infection should be considered in patients with prolonged febrile illnesses of obscure etiology

Human infection with Yersinia enterocolitica a small gramnegative rod of genus Yersinia has been recognized as a frequent cause of human disease during the last decade Commonly observed are acute or subacute gastroenteritis mesenteric lymphadenitis erythema nodosum and arthritis (1 3 11 12 13 19) However quite a variety of other pathological conditions has been associated with yersiniosis as carditis (1 12) acute hepatitis and glomerulonephritis (4 12) Reiter s disease (17) skin manifestations (8 11) eye inflammations (2) meningitis (18) and thyroid disorders (5)

This paper presents a case of yersiniosis with prolonged monosymptomatic fever of a hectic type as the only clinical manifestation

CASE REPORT

A 14 year-old girl with no significant diseases in her past was admitted to our department due to one week s fever of unknown origin During that period she had been in bed with normal to slightly elevated temperature in the mornings and fever around 40 C in the evenings She had had no sore throat respiratory symptoms diarrhoea abdominal pains arthralgias or skin eruptions and clinical examination by her general practitioner disclosed normal findings There had been no sickness in her surroundings It was later revealed that she had been in close contact with a chinchilla 4–5 days before her fever erupted but neither this animal nor its host family had demonstrated signs of illness at that time

After admission she was kept in bed and her fever persisted as at home that is a hectic type of temperature curve (Fig 1) Apart from slight tiredness and intermittent headache during the periods of elevated temperature she had no other symptoms whatsoever

Her clinical appearance was that of a normal healthy girl except for slight flushing of her cheeks related to high temperatures Physical examination was performed by several doctors who agreed upon negative findings ESR 50–60 Hb 12 5 g/100 ml BP 120/80 normal electrolytes Urinary sediment ECG X rays of thorax sinuses and teeth eye examination and gynaecological exploration revealed nothing abnormal WBCs were persistently normal except for slight relative lymphocytosis after two weeks of fever Ten subsequently cultivated venous blood samples were negative

Extensive laboratory investigation was done to reveal the etiology of her hectic fever She had normal thrombocytes liver tests and cold agglutinins LE cell test TB test antinuclear and rheumatoid factors were negative Electrophoresis of the serum proteins showed slightly elevated α_2 globulins and her immunoglobulins were within normal ranges Serological testing for leptospirosis mononucleosis ornithosis syphilis toxoplasmosis and infection with gonococci Mycoplasma pneumoniae and haemolytic streptococci was negative Nothing abnormal was found at Widal s agglutination reactions to Salmonella typhosa B abortus Bang S typhimurium and paratyphi B H antigen Paratyphi B O antigen showed 1 50 but this value was unchanged during and after her disease

A consultant in clinical microbiology favoured a viral etiology of her severely fluctuating fever with normal WBCs and good clinical condition An advocated tetra

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Bilateral Atrial Myxomas Diagnosed by Echocardiography

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ABSTRACT Bilateral atrial myxomas have been demonstrated with the aid of echocardiography in a 31 year old male patient The diagnosis was confirmed by angiography and the tumors were removed successfully at operation

Since the first report by Effert and Domanig (4) the value of echocardiography in the diagnosis of left atrial myxoma has been confirmed by a rapidly increasing number of papers in recent years (9 11 17 18 19 21 23 ?7) Myxomas more often originate from the left atrium than from the right the ratio being about three to one (2?) There have also been a few reports concerning the diagnosis of right atrial myxoma by echocardiography (5 7 10 12 ?6 27) Bilateral atrial myxomas are very infrequent (29) the diagnosis was established by echocardiography in one case (?9)

We report a patient with bilateral myxomas who to our knowledge constitutes the second case diagnosed by echocardiography

CASE REPORT

The patient a 31 year-old man was admitted in Oct 1973 The history revealed that symptoms had begun 12 years before admission with *constitutional symptoms* including periods of fever and muscle pains signs of pericarditis and pleuritis elevated ESR and increased serum γ-globulin Signs of *embolism* appeared in 1963 with renal symptoms followed by recurrent neurological symptoms and signs of obstructions of the arteries of the lower limbs *Heart murmurs* suggestive of mitral valve disease had been observed since 1961 An ECG in 1966 showed signs of an inferior wall myocardial infarction Angiographic studies demonstrated aneurysms in the cerebral renal and popliteal arteries A diagnosis of polyarteritis nodosa and rheumatic heart disease was first established the clinical course has been reported in detail (14 15) When an echocardiogram aroused suspicion of a left atrial tumor the patient was admitted to the Department of Cardiology University Hospital Lund

On admission auscultation of the heart revealed a presystolic murmur a loud first heart sound a short high pitched systolic and a low pitched early diastolic murmur at the apex No diastolic sound was audible ECG showed sinus rhythm and sequelae of an inferior wall myocardial infarction

Echocardiography was performed with a Smith Kline instrument (Echoline 20) at a frequency of 2 25 MHz Tracings were recorded with the aid of a polaroid camera mounted in front of a Tektronix oscilloscope Fig 1*a* shows that multiple echoes appeared behind the anterior mitral leaflet during diastole an echo-free space was seen to the right of the leaflet during the opening movement in early diastole The transducer was then rotated in such a direction that echoes were obtained from the tricuspid valve Abnormal echoes with a laminated appearance were observed behind the anterior tricuspid leaflet during both systole and diastole (Fig 1*b*) Fig 1*c* shows a recording of the echoes when the direction of the transducer was changed from the tricuspid to the mitral valve

Angiocardiography was performed by percutaneous catheterization of the right femoral vein When contrast was injected into the right atrium lobulated filling defects appeared above the tricuspid valve they floated down into the right ventricle during diastole The emptying of contrast from the right atrium was normal A second injection of contrast into the pulmonary artery revealed a lobulated filling defect in the left atrium with an estimated diameter of 4 cm The tumor moved into the left ventricle during diastole and back into the left atrium during systole The mean pressures in the right atrium and in the pulmonary artery were normal

At operation gelatinous friable tumor masses welled from the right atrium when the atrial wall was incised These tumor masses were sucked out and the base of the tumor which originated from the interatrial septum was excised The left atrium contained a tumor of a similar size and consistency originating from the left side of the septum The tumor was removed and the septum was closed without a patch The tricuspid and mitral valves were normal Microscopic examination of the tumors showed them to be myxomas The postoperative course was uneventful An echocardiogram showed no abnormal echoes (Fig ?)

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Partial Correction of Hypertension by Angiotensin II Blockade in a Patient with Phaeochromocytoma

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ABSTRACT This case report describes a patient with malignant hypertension and phaeochromocytoma in whom blockade of angiotensin II receptors by the competitive antagonist 1 sar 8-ala angiotensin II (Saralasin®) resulted in a partial correction of the elevated BP Plasma renin activity was high and rose further during the blockade Competitive inhibition of angiotensin II by Saralasin does not abolish the pressor effect of catecholamines It was therefore interesting to observe that in this patient with phaeochromocytoma independently both α adrenergic receptor blockade and angiotensin II receptor blockade were effective in lowering BP

The catecholamine and the renin–angiotensin–aldosterone system are interrelated angiotensin II and some of its analogues can increase sympathetic activity (14) and the sympathetic nervous system probably plays an important role in the regulation of renin release (3 11 18 20)

It is generally assumed that in patients with phaeochromocytoma hypertension is a consequence of the vasoconstrictive action of catecholamines circulating in excess (12) However patients with phaeochromocytoma are also reported to have increased plasma renin activity (PRA) and plasma aldosterone levels (7 9 13 19 21 22) which normalized after α receptor blockade (19) It is not known whether the stimulated renin–angiotensin–aldosterone system contributes to the high BP in these cases This can be studied by a blockade of the system for instance by administering 1 sar-8 ala angiotensin II (Saralasin®) a competitive antagonist of angiotensin II (2 4)

This case report describes a patient with phaeochromocytoma and malignant hypertension in whom the hypertension was partly corrected both by α adrenergic receptor blockade and by angiotensin II blockade The possible explanations of the phenomenon in this particular case are discussed

CASE REPORT

The patient was a 46-year old man who had suffered from attacks of headache for seven years In Feb 1975 his vision became blurred At that time a BP of 280/170 mmHg and a grade IV hypertensive retinopathy were found Serum sodium was 145 mEq/l serum potassium 4 3 mEq/l and serum creatinine 1 0 mg/100 ml I v pyelography disclosed a large tumour mass above the right kidney displacing the kidney caudally (Fig 1) A regimen of sodium restriction and clonidine 3×0 15 mg/24 hours was instituted One week afte the start of this therapy the patient was admitted to a hospital elsewhere because of vomiting BP was 250/140 mmHg Clonidine was discontinued and the following treatment was instituted sodium restriction epithiazide (40 mg) triamterene (50 mg) α methyldopa (2 g) and propranolol (170 mg) daily Despite this regimen BP remained high Serum creatinine rose from 1 9 mg/100 ml on the third day after admission to 4 0 mg/100 ml on the fifth The patient became disoriented and drowsy Because of renal failure he was transferred on the seventh day to the University Hospital in Groningen On this day the antihypertensive drugs were withdrawn

On physical examination the patient was hardly responsive His peripheral circulation was contracted BP was 180/140 mmHg pulse rate 96/min and temperature 37 7°C Hb was 16 6 g/100 ml leucocytes 18 000 mm³ with a normal differential count and platelets 309 000/mm Serum sodium was 1 8 mEq/l serum potassium 5 2 mEq/l serum urea 440 mg/100 ml and serum creatinine 4 8 mg/100 ml Fundoscopy revealed a grade IV hypertensive retinopathy and on neurologic examination a hypertensive encephalopathy was diagnosed A chest X-ray show



F g 1 I v pyelogram show ing a large tumour mass above the right k dney d plac ng th s k dney cauda ly (Placed at our d posal by Professor Dr J R B k man)

enlarged heart wh le on ECG left ventricular hypertrophy and a stra n pattern were noted

Suspect ng a phaeochromocytoma 7 5 mg of phen *tolam ne were injected i v and after 3 m n th s resulted* in a lowering of the BP to 90/85 mmHg At th s po nt it was reasoned that th s hypertens on could at least n part be due to st mulat on of the ren n-ang otens n system caused by the d splacement and consequent funct onal renal artery steno s of the right k dney Therefore ten hours after the phen olam n test i v adm n strat on of as n* (Eaton Laboratories Norwich Benelux) was rted n a do e of 1 5 μg/kg/m n and BP was measured automa cally every 2 m n (F g 2) After 2 m n of infus on BP had decrea ed from 200/130 to 125/90 mmHg and f r fear of hypoten on Saralas n adm n strat on was temporar ly d cont nued Two m nutes later BP had in creased t 200 160 mmHg and Saralasin infus on was re n st tuted in a dose of 6 μg/kg/m n result ng in an immed ate d op of the BP stab l z ng around 150/100 mmHg on a Saralas n do e of 3 μg/kg/m n In o der to control BP thereafter in reas ng do e of Saralas n (6–8 μg/kg/m n) were needed Saralas n was then comb ned w th i v phenoxybenzam ne and p opranolol n doses shown in F g 2 BP was kept under control by comb ned α and β receptor blockade al ne although there was a tempo rary sharp in rea e in BP upon d cont nuat on of the Saralas n med cat on (F g

During ang otens n II blockade by Saral is n d ures s together w th sod um and creat n ne ex e ion decreased (F g 3) Pre nfus on value were reached after withdraw ng Saralas n The already h gh PRA le el 2900 ng A / 10 ml/3 hours rose to 14600 during Sarala n nfus on (Normal upper value in sup ne pos t n and on a sod um intake of 100 mEq 24 hou am unt t 105 ng/A /10 ml/3 hours) Af er d scont nuat n of Sa ala n wh le the pa t ent was rece v ng comb ned α and β receptor blockade PRA returned to the in tial pre Saralas n value (F g 3 The PRA assays were performed by Dr H J G Holle mans

On the following days cont nued phenoxybenzam ne and propranolol therapy kept BP around 160/100 mmHg The pat ent rega ned consciousness and the s gns of hypertens ve encephalopathy d sappeared However the cl n cal course was compl cated by a double s ded a p ra t on pneumon a requir ng intensive care surve llance and art f cial resp rat on On the 14th day of hosp tal zation card ac fa lure developed suddenly and the pat ent d ed

A t ps

The right kidney was d splaced caudally by a tumour measuring 10 cm in d ameter obv ously originat ng from the right adrenal gland Neither the right renal artery and ve n nor the right ureter seemed to be ob tru ted by the d placement of the k dney (Fig 4) The tum ur wa round and well encapsulated A remnant of the righ adrenal gland was stretched over its convex ty B h the gross and the m cro cop c appearan e conf rmed the clini cal d agnos s of phaeochromocytoma Noradrenal ne wa shown in the cells in frozen sect ons f f rmal n-f ed t ssue by autofluorescence (15) The left adrenal gland was normal The k dneys we ghed 165 g each and showed no gross abnormal t es Both k dneys had the same m croscop c appearan e sl ghtly schaem c gl merular tufts w th wrinkled basement membranes some a roph of the tubulus l ttle interst t al oedema and venous con gest on The ma n changes were found n the arteries of both k dneys sub nt mal hyal n depo n th cken ng of the med a and areas of nt mal fibros Necros s of he med a could not be dem nstrated unequ voca ly A f w fibr n depo ts were seen in glomerular cap llaries The same vacular changes were found in o her organs and th ught to be due to hypertens on The juxtaglomerular

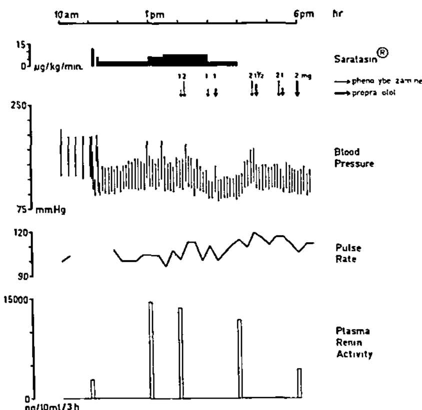


Fig 2 Effect of angiotensin II and α and β-adrenergic receptor blockade on BP pulse rate and PRA Each vertical line represents the mean of 3 BP recordings

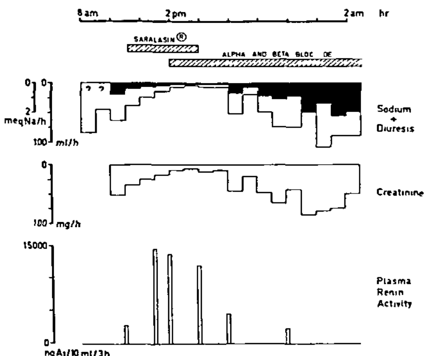


Fig 3 Effect of angiotensin II and α and β-adrenergic receptor blockade on diuresis sodium and creatinine excretion and PRA



F g 4 Posterior v ew right k dney d splaced by a phaeochromocytoma of the right adrenal gland no ob truc t on of the r ght renal artery

apparatuses were nconsp cuous and of the same s ze in both k dneys

The lungs showed the p cture of a severe bronchopneumon a w th a protracted course due to art f c al resp rat on The left ven cle was hypertroph c as a con equence f hyperten on The thyro d was normal w th negat ve sta n ng react ons for amylo d and h stopatholog cal exam nat on revealed no C cell hyperplas a

DISCUSSION

The p tient descr bed in th s report presented phaeochromocytom   m lign nt hypertens on and increased PRA in wh ch angiotensin at least ulti*mately c ntr buted to the m ntenance of the ele*vated BI   Alth ugh it has been shown that in cre sed levels of c techol mines c n be respons ble for an elevated PRA in some patients with phaeo chromocytoma (19) other poss b l ties also have to be considered to expl n both the partial correction of the hypertension by angiotensin II blockade and the elevated PRA in this particular case

Correction of hypertens on by Saral sin* w ll be influenced by an existing sympathetic nh b ti n (6) and by sod um depleti n (5) In the present case both factors can h ve played a role in the sensitivity to the hypotensive act   n of Saral s n especially when angiotens n II α and β blocka le were com bined However an incre sed sen tiv ty t Saral s n does n t expla n why PRA was elevated before ang otensin II blockade h d been started It is pos s ble that the latter resulted from the mal gnant phase of the hypertens on itself probably ag gravated by the sodium restrict on and the ad m n strat on of diuret cs   s nce this reg men w l further reduce a pl sma volume th t   n pat ents with phaeochromocytoma is already subnormal (12) It is well kno vn that hyponatriaem a also in creases ren n release through a tubular mechan m relat ng to decreased filtered sodium loads (17) Moreover it has been shown that clon d ne n creases PRA in p tients with phaeochromocytoma (8) It is noteworthy in th s respect that the k dney funct on of our pat ent deteriorated after the *start of ant hypertens ve therapy An increased* PRA due to d splacement of the k dney and consequent funct on l renal artery steno s seems unlikely   since no d fference was f und between the left and the r ght kidney in hypertrophy of the juxtaglomerular apparatuses or in the severity of the les ons of the small ren l arteries

Both α adrenergic and ang tensin II recepto blockade lowered BP in th s p tient The v ew that both systems have the same receptors is hard to accept   s nce Sar las n is un ble to bl ck the pres s r effect of noradrenal ne (7) Furthermore the positive phentolam ne test cann t be expla ned either by interference with the d rect vasoconstr t ve action c f ang otens n II (1) c r by suppress   n of ng c tensin product on   as the half l fe of ren n i about 45 m n (10) Hence one has to c nclude tha

ultimately catecholamines as well as angiotensin II sustained the elevated BP in our patient A similar observation has been made previously in a patient with unilateral renovascular hypertension complicated by hyponatriaemia and a plasma volume at the lower limit of normal i v phentolamine resulted in a sharp decrease in BP in that patient probably because sodium depletion also causes increased sympathetic activity (1) In support of our supposition was the observation that withdrawal of the analogue resulted in an increase in BP despite continuing α and β blockade Moreover the BP was normal when Saralasin was administered together with phenoxybenzamine and propranolol while the latter two drugs kept BP under control notwithstanding a persistently high PRA

The competitive action of Saralasin for angiotensin II was illustrated by the increasing amount of the analogue necessary to control BP as more angiotensin II was generated by a stepped up production of renin The reason for this may be found in the decrease in BP but also in a blockade of the direct negative feedback between angiotensin II and renin by Saralasin (16) Saralasin may be antagonistic to angiotensin II on BP and it may be agonistic on renal function as expressed in this case by a decrease in diuresis and in sodium and creatinine excretion during the infusion Comparable data obtained in patients with unilateral renovascular hypertension indicated a correlation with a decrease in the effective renal plasma flow (5)

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BOOK REVIEW

Handbook of Hemophilia Edited by K M Brinkhous and H C Hemker 2 volumes 927 pp 142 tables 289 illustrations US$135 50 American Elsevier Publishing Company Inc New York 1975

This book was written at the suggestion of the late Professor S van Creveld in Amsterdam who was one of the pioneers in hemophilia research and the book begins with a commemorative article on his work This extremely comprehensive text book comprises eight major sections with 63 chapters by 89 authors representing disciplines dealing with the basic and clinical problems of hemophilia A hemophilia B von Willebrand s disease and their variants and complications The authors have been selected mainly from Professor van Creveld s school in Holland and from the staff of Professor Brinkhous in Chapel Hill

The first section contains a historical and general outline of the nature of hemophilia and the hemostatic mechanism It also contains a chapter on »The incidence of hemophilia in South Africa This chapter appears superfluous The frequency and incidence of hemophilia in various countries have been studied in detail by several workers and especially from Scandinavia

The second section consists of 14 chapters on biochemistry pathophysiology genetics and the prospects of transplantation in hemophilia The chapter by Wagner and Cooper of Chapel Hill and that by van Mourik Bouma and Mochtar on the biochemistry of factor VIII are first class The chapters on molecular variants of factor VIII and on von Willebrand s disease are no longer up to date The chapter by Roberts and Cederbaum on molecular variants of factor IX is of interest but here again many of the latest events are missing For instance the work by Stenflo (1973) on vitamin K dependent modifications of glutamic acid residues in prothrombin are lacking as is the work by Østerud on electroimmunoassay of coagulation factor IX One chapter on transplantation prospects in hemophilia would have been sufficient Moreover it is astonishing that the editors were so tolerant For example one author uses the abbreviation AHF another AHG The references in the various chapters are not given in a uniform way In all chapters there is a considerable overlap and the contents of the book would have been clearer if it had consisted of only one first class review of each subject Dr Graham has written an excellent article about genetics Dr Veltkamp has presented a very short article about carriers Here it should be pointed out that factor VIII related antigen increases in all reactive processes and thereby creates a considerable source of error in the evaluation of the ratio between F VIII C and F VIIIR AG Professor Verstraete has reviewed literature cases of hemophilia combined with other congenital deficiencies Though such cases are rare it is useful to have them summarized

Section C includes 11 chapters on pathology and clinical aspects Drs Astrup and Brakman have written about tissue repair and vascular disease in hemophilia Their article is somewhat disappointing For example it is claimed that intracranial hemorrhage is rare in hemophilia though it is now common knowledge that intracranial hemorrhage is the most frequent cause of death in hemophilia It is puzzling to read that the finding of a relation between the severity of the coagulation defect and the clinical manifestations is unique There is a fair amount of overlap of the articles dealing with the clinical aspects of various bleeding manifestations Yet they do not give anything like a complete picture of the bleeding manifestations of hemophilia One wonders for what readers these chapters are intended A beginner in the field cannot learn the clinical details of hemophilia from th m and they contain nothing new to specialists

Section D deals with the preparation and therapeutic use of factor VIII and factor IX concentrates There are special chapters on high potency human factor VIII concentrates cryoprecipitate fraction I-0 and F I-0-a and two chapters on factor IX concentrates Several of these chapters are very well written but very detailed and most of them have been presented in earlier publications The thrombogenic complications of factor IX concentrates are only briefly mentioned There is also a fair amount of unnecessary repetition The chapters on home therapy and those on complications of therapy and on hepatitis are good and useful

Section E surveys the inhibitor problems The use of activated prothrombin complex concentrate is only briefly mentioned

The next section which consists of 6 articles concerns general and supportive therapy Some of them are good but the choice is poor Thus nothing is said about treatment in association with general or orthopedic surgery In view of the limited substitution therapy given the results in the paper on synovectomy are astonishing The dental management has received a disproportionately large space and is the subject of as many as 5 articles This is not good editorial work

The book ends with a section on social aspects

It is an unusually large textbook on hemophilia The section on genetics and biochemistry is the best The clinical parts are of varying value and should have been edited better The book may be of value to investigators in the field of hemostasis but hardly as a textbook for beginners in the field

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EDITORIAL

Myelopoiesis in Human Disease

The number and the types of hemopoietic cells in the peripheral blood of healthy individuals are strictly defined This simple observation has led to the postulation of humoral regulatory mechanisms for the blood cells Erythroid cells were the first to be studied extensively Although some problems re main to be solved considerable knowledge has been accumulated both on erythroid cell prolifera tion and maturation and on the hormone erythro-poietin responsible for this regulation Similar studies of mechanisms involved in the control of WBC production were hampered by the lack of suitable assay systems One of the problems was the lack of a specific label for white cells on the lines of ^{59}Fe for the red cells Other problems were that a considerable part of the peripheral granulo cytes is marginal rather than circulating and the presence of a large bone marrow pool For these reasons it seemed clear that studies of hormonal regulation of myeloid cells could not easily be con ducted in vivo but awaited the development of in vitro assay systems A technique for the culture of animal bone marrow myeloid cells was introduced in 1966 by Bradley and Metcalf in Australia and Plutznik and Sachs in Israel A modification of the technique by Robinson and Pike in 1970 made it possible to grow human bone marrow cells and focused even more attention on the research poten tial of this field From the large number of publica tions in recent years a new picture has emerged of the cellular and humoral events in myelopoiesis both in health and disease The aim of this paper is to introduce the various aspects of this field of research

Culture techniques (1 2 7)

The first method described was the in vitro culture of mouse bone marrow cells where myeloid stem cells proliferate and give rise to colonies of mature granulocytes and macrophages in a matrix of semi solid agar Colony formation in vitro depends on the presence of a humoral substance termed the colony stimulating factor (CSF) For the culture of human bone marrow cells a double layer agar technique has to be used where the necessary stimulus is provided by a bottom layer of human peripheral blood cells Modifications of these basic techniques have been developed involving for instance the use of methyl cellulose instead of agar as a support ing substance Bone marrow cells have also been cultured in liquid medium and in diffusion chambers implanted into the peritoneal cavity of mice

The colony forming cell (6 7 9)

The colonies of myeloid cells are each derived from a single precursor stem cell more primitive than the myeloblast but committed to the myeloid path of differentiation a so-called unipotential stem cell These cells give rise to either granulocytic or macrophage colonies implying a close relationship between the two cell types Cell separation studies suggest that the colony forming cell (CFC) is a large mononuclear cell with a leptochromatic nucleus and basophilic cytoplasm that on routine bone marrow smears would be classified as a transitional lymphocyte In acute myeloid leukemia CFC has been found to be of abnormally light density with a return to the normal density in remission

Colony stimulating activity in serum and cells (3 10 11)

CSF can be extracted from a number of organs and may also be found in serum and urine Chemi cal characterization has shown that the human urinary CSF is a glucoprotein with a molecular weight of 45000 daltons i e physico-chemically similar to erythropoietin Increased levels of CSF have been found in serum and urine from patients with leukemia in acute infections and in experi mental animals after endotoxin injection or irradia

tion This activity may be masked by the presence of inhibitors in serum or urine (see below) As highly purified preparations of CSF are still available in small amounts only the evidence for the physiological role of the hormone is indirect However injections in mice of partially purified urinary CSF have been shown to increase peripheral blood neutrophil counts Infusion of plasma with a high CSF content in a patient with neutropenia increased peripheral granulocyte counts These experiments raise the hope that purified CSF when available in large amounts could be of therapeutic value

The *colony stimulating activity* (CSA) produced by human peripheral blood cells which are necessary for the proliferation in vitro of human marrow cells is chemically less well defined than the urinary CSF Cell fractionations of peripheral blood cells have shown that this stimulating factor is produced by the monocyte macrophage cells The ubiquitous presence of these cells in the body has led to an attractive model for granulopoiesis Increased CSA production by the monocyte macrophage system in response to a foreign antigen would result in an increased granulocyte production to meet the demand in for instance bacterial or viral infections

This model of granulopoiesis is still hypothetical and leaves several questions unanswered Some kind of feedback control has to be postulated and both a negative and a positive model have been suggested There has been a considerable search for *inhibitors* of granulopoiesis and at least two types of inhibitory factors have been described Serum lipoproteins belonging mainly to the light density lipoprotein class have been shown to inhibit colony formation in vitro Small inhibitors produced by mature granulocytes have also been suggested to inhibit granulopoiesis but much experimental work remains to be done in this field

Myeloid leukemias and preleukemias (1 3 4 5 8 9)

Both bone marrow cells and peripheral WBC from patients with acute myeloid leukemia (AML) have been shown to have an impaired capacity to form colonies in vitro Some patients cells altogether fail to form colonies or form abnormally small colonies or clusters Cytogenetic studies have demonstrated that most if not all of these clusters are of leukemic origin There is evidence that the occurrence of colonies of certain cultural patterns is of prognostic value as to the likelihood of achieving remission In bone marrow culture formation of small clusters without colonies or formation of colonies in association with the normal number of clusters seems to be associated with a high probability of remission The occurrence of both CFC and CSA in culture of peripheral blood cells seems to have a similar correlation to prognosis Complete remission induced by cytotoxic drugs is reflected in vitro by an apparently normal colony growth pattern Relapse is associated with a return of the leukemic growth pattern in many cases preceding the clinical picture by weeks At least three important conclusions emerge from the in vitro culture results 1) Leukemic cells are capable of at least partial differentiation (cluster formation) 2) Cluster and colony formation by leukemic cells is dependent on the presence of a stimulating factor i e leukemic cells are still responsive to and stand under hormonal control 3) Serial cultures may help in monitoring therapy and evaluating remission status

Colony formation by the marrow or peripheral blood in untreated chronic myeloid leukemia is normal or especially in the peripheral blood greatly above the normal level These apparently normal colonies are of leukemic origin since the Ph' chromosome is found in a majority of them Treatment of the patient reduces the number of colonies to normal or subnormal levels Blastic transformation is accompanied and often preceded by a reduction of colony numbers and a change to an AML type of colony-cluster growth pattern

Culture of cells from patients with different preleukemic myeloproliferative disorders has given variable results mostly reflecting different definitions of the preleukemic state But when preleukemia has been defined as an unclear clinical state that eventually develops into overt leukemia the pattern has been remarkably consistent whatever the culture technique In a majority of cases the CFC had an abnormally light density the bone marrow growth in vitro was of the leukemic type and this growth abnormality could be noted weeks in some cases months before the clinical diagnosis of leukemia could be confirmed morphologically

Neutropenia (1 2 4 9)

The in vitro culture techniques have been used to study neutropenic disorders but no consistent pattern has been established The variation seems

to reflect differences in the pathophysiology of the disorders leading to neutropenia In myeloid hypoplasia and aplastic anemia there have been decreased numbers of CFC in the marrow In Felty s syndrome CFC were either increased or normal Idiopathic neutropenia has given variable results which suggests a heterogeneous etiology In cyclic neutropenia both CFC and CSF showed a cyclic variation—CFC in the marrow and CSF levels in serum and urine both varied inversely with peripheral neutrophil counts and directly with the monocyte count In infantile genetic agranulocytosis both normal marrow CFC with a normal in vitro maturation and normal marrow CFC with defective in vitro maturation (maturation arrest at the promyelocyte stage) have been reported Low marrow CFC has been found in drug induced neutropenia and the decrease was dose dependent

The experimental model of myelopoiesis the humoral regulators and the clinical applications of the in vitro culture technique cited above are just a few examples of trends in a rapidly expanding field of research The hypothetical scheme for the control of granulocyte and monocyte production is being developed and modified continuously by recent results and the in vitro culture technique is being applied in new fields These efforts will hopefully serve to increase our knowledge of granulocytopoiesis in health and disease so that more specific therapeutic programs can be devised for the treatment of hemopoietic disorders

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Prognostic Value of Colony-stimulating and Colony-forming cells in Peripheral Blood in Acute Non-lymphoblastic Leukemia

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ABSTRACT Colony forming cells (CFC) and colony stimulating activity (CSA) in peripheral blood cells have been studied before and repeatedly during treatment of 30 patients with acute non lymphoblastic leukemia WBC obtained after Isopaque-dextran separation were cultured in vitro by a double-layer agar technique Before treatment 16 patients out of 30 had CSA and 22 out of 29 had CFC, both CSA and CFC were found in 15 patients In follow up studies during treatment CSA was mainly unaffected during the leukopenic phase, while CFC were suppressed No CFC were found at WBC counts below 900/mm^3 This seems to imply that CFC are more sensitive to cytotoxic agents than colony stimulating cells Twelve patients entered remission all of them had CSA and all the 11 who were investigated for CFC had CFC before treatment Fourteen out of 18 non responders lacked one or both types of cells The presence of CSA and CFC in peripheral blood therefore appears to be a sign of favorable prognosis, while the absence of CSA and/or CFC implies lack of response to treatment

It has been difficult to find clear clinical or hematological criteria for the prognosis in acute leukemia Parameters such as total WBC count percentage of blast cells and platelet count are generally of limited value in predicting response to therapy

The in vitro cultivation of human bone marrow and peripheral blood cells in semi solid medium (3 14 18 19) has proved to be a valuable method for investigating defects of myelopoiesis in myeloid leukemia In a large patient population of acute non lymphoblastic leukemia Moore et al (15) established a classification system according to in vitro growth characteristics of bone marrow cells They also found prognostic implications As reported earlier from our laboratory (10 13) there seems to be a relation between a good prognosis and the presence of colony forming cells (CFC) and colony stimulating cells (CSC) in the peripheral WBC population The aim of the present study was to further characterize this proposed relationship

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PATIENTS

Thirty adult patients (9 males 21 females) with acute leukemia were investigated 27 had acute myeloblastic (AML) and 3 acute undifferentiated leukemia (AUL) Their ages ranged from 22 to 83 years (median 57) At the time of the first sampling for CFC and CSC all patients were untreated (except for blood transfusions in 8) Patients under 70 years were given induction treatment with rubidomycin (1.5 mg/kg b wt for one day) and cytosine arabinoside (1 mg/kg b wt ×2 for 5 consecutive days) in intermittent courses with a 5-day free interval (20) Patients over 70 were treated with intermittent courses of cytosine arabinoside (80 mg/m^2 daily for 5 days) and thioguanine (70 mg/m^2 daily for 5 days after a free interval of 1 day) to be repeated every 21 days (6 12)

Twelve patients entered complete remission To maintain remission patients under 70 received rubidomycin + cytosine arabinoside and thioguanine + cytosine arabinoside in alternate courses (20) In addition nine of the 12 patients in remission received immunotherapy (weekly bacillus Calmette-Guerin preparation and allogeneic leukemic cells) (4 6) Fifteen patients were sequentially studied during treatment

Healthy persons were investiga atedly and simultaneously on the same occ kemic patients

Table I *Pretreatment data on 30 leukemic patients*

CFC=colony forming cells CSA=colony stimulating activity (mean no of colonies/10^6 peripheral WBC) AML=acute myeloblastic leukemia AUL=acute undifferentiated leukemia n d =not done

Pat no	Sex	Age (y)	Diagnosis	Total WBC/mm³	Blast cells (%)	Platelets ×10³/mm³	CFC	CSA	Complete remission
1	♀	67	AML	56 000	97	10	0	0	–
2	♂	58	AML	48 000	40	20	0	0	–
3	♂	81	AML	161 000	91	44	0	0	–
4	♀	70	AML	22 000	90	52	0	0	–
5	♂	55	AML	500	0	121	0	0	–
6	♀	50	AML	780	23	225	0	0	–
7	♀	79	AML	153 000	80	15	0	0	–
8	♀	72	AML	96 000	84	62	9	0	–
9	♂	83	AML	51 000	86	10	5	0	–
10	♂	60	AUL	16 400	10	3	12	0	–
11	♀	58	AML	2 700	13	25	7	0	–
12	♂	28	AUL	4 500	0	11	4	0	–
13	♀	47	AML	2 000	40	14	5	0	–
14	♀	56	AML	1 900	80	10	12	0	–
15	♀	66	AML	48 500	59	43	11	7	–
16	♀	48	AML	160 000	53	10	4	1	–
17	♀	68	AML	20 700	66	108	18	17	–
18	♀	63	AML	5 000	81	69	5	2	–
19	♀	64	AML	2 600	3	67	1	2	+
20	♀	52	AML	19 600	85	39	18	6	+
21	♀	35	AML	4 500	53	134	2	4	+
22	♀	56	AUL	3 500	11	81	13	6	+
23	♂	36	AML	11 100	3	227	71	95	+
24	♀	68	AML	22 000	7	16	55	16	+
25	♀	74	AML	58 000	50	75	2	2	+
26	♂	41	AML	2 600	0	64	3	2	+
27	♂	39	AML	75 000	10	61	n d	10	+
28	♀	69	AML	38 400	38	49	26	18	+
29	♀	54	AML	80 000	16	23	4	4	+
30	♀	22	AML	19 700	11	46	49	28	+

CULTURE TECHNIQUE

Venous blood 10–20 ml with 20 IU/ml of preservative free heparin was collected from leukemic patients and healthy donors Separation of WBC from heparinized blood was done in Isopaque-dextran as described by Boyum (5)

The two layer agar cultivation method of Robinson et al (18 19) was used without modifications The culture medium consisted of modified McCoy s 5A medium with 15% fetal calf serum (19) The medium was mixed with agar to a final concentration of 0 3% agar in the over layer and 0 5% in the feeder layer WBC obtained by Isopaque-dextran sedimentation were carefully washed in the medium and adjusted to a final concentration of 1×10^6 cells/ml in the feeder layer as well as in the over layer One ml of the feeder layer mixture followed by one ml of the over layer mixture were then poured into 35 mm Falcon Plastic petri dishes Cultures were incubated in a humidified atmosphere at 37 C and 10% CO_2 in air After 14 days of incubation colonies consisting of more than 20 cells in the over layer were counted in an inverted microscope

CFC in the peripheral blood from leukemic patients were studied by plating their WBC over a feeder layer of WBC from healthy persons Colony stimulating activity (CSA) in the peripheral blood was studied by using WBC from the leukemic patients in the feeder layer with cells from healthy controls as indicator cells in the over layer Control cultures of peripheral WBC from the same healthy subject in both the feeder and the over layer were set up in each experiment

RESULTS

Colony forming cells

CFC were found in the peripheral blood of all controls A mean of 13 2 colonies/10^6 cells (range 1–39) was obtained in the over layer when cells from the same healthy donor were plated in the feeder layer

Twenty two out of 29 patients with acute leukemia had CFC before any treatment was given (Table I) information is lacking on one patient The mean number of colonies was 15 3/10^6 cells in these 22 patients i e similar to the colony forming

Table II *Colony forming cells (CFC) and colony stimulating activity (CSA) (no of colonies/10^6 WBC) before and during treatment in 15 patients*

Pat no	Time of sampling (d after diagnosis)	Total WBC/mm³	Blast cells (%)	CFC	CSA	Complete remission during therapy	Survival after diagnosis
2	0	48 000	40	0	0	−	3 mo
	13	3 000	0	0	0	−	
	40	11 000	7	0	0		
4	0	22 700	90	0	0	−	3 mo 10 d
	35	6 000	98	0	1		
	48	1 600	56	0	2		
8	0	96 000	84	9	0	−	51 d
	9	7 200	84	2	0		
	37	3 000	18	1	0		
9	0	51 000	86	5	0		40 d
	9	10 400	91	2	0		
	37	12 000	94	1	0		
19	0	2 600	3	1	2	+	1 y 2 mo
	7	1 200	0	16	3		
	60	900	0	0	1		
	120	4 200	0	2	1		
	150	3 290	0	2	1		
	215	2 900	0	1	1		
20	0	19 600	85	18	6	+	1 y 8 mo
	10	1 800	51	6	6		
	50	2 700	0	8	9		
	70	4 100	0	2	1		
	116	4 900	0	1	1		
	150	3 500	0	1	n d		
	450	4 400	0	6	n d		
21	0	4 500	53	2	4	+	>3 y 3 mo
	20	900	0	0	15		
	102	4 500	0	5	4		
	500	5 400	0	9	n d		
22	0	3 500	11	13	6	+	1 y 5 mo
	28	680	0	0	2		
	68	8 800	0	2	3		
	375	6 900	0	10	n d		
10	0	16 400	10	12	0	−	3 mo
	8	2 400	32	224	7		
	66	1 200	0	7	0		
5	0	500	0	0	0	−	38 d
	18	1 100	5	1	0		
23	0	11 100	3	71	95	+	>2 y 1 mo
	40	800	0	0	1		
	135	3 200	0	18	14		
	383	1 000	0	7	6		
24	0	22 000	7	55	16	+	>1 y 8 mo
	60	8 200	0	59	22		
	360	7 600	0	8	n d		
	422	6 700	0	5	16		
12	0	4 500	0	4	0	−	6 mo
	40	800	0	0	15		
	120	400	0	0	1		
13	0	2 000	40	5	0	−	2 mo 17 d
	30	800	22	0	2		
25	0	58 000	50	2	2	+	7 mo
	51	4 300	0	5	6		

n d = not done

Table III *Colony forming cells (CFC) and colony stimulating activity (CSA) in peripheral WBC before treatment related to prognosis in patients with non lymphoblastic leukemia*

	No of colonies/10^6 cells			
	0	1–5	6–20	>20
CFC in 29 patients				
Complete remission	0	5	2	4
Without remission	7	5	6	0
CSA in 30 patients				
Complete remission	0	5	5	2
Without remission	14	2	2	0

Patients with CFC and entering remission differed from those not entering remission at the level of $p<0.02$ (χ^2 test) Patients with CSA differed from those not entering remission at the level of $p<0.001$ (χ^2 test)

capacity of normal cells However the range (1–71 colonies) was considerably wider than in the normals the number of colonies in three patients being well above the range of CFC in normal cells Excluding these patients the mean number of CFC in the other 19 was 8 5 indicating a lower CFC initially in most leukemic patients than in normal individuals (Table I)

The CFC usually persisted during treatment They disappeared however during pronounced leukopenia induced by the cytotoxic drugs No CFC were found at WBC counts below 900/mm³ in six patients during treatment (Table II)

No statistically significant correlation was seen between the initial presence of CFC and any of the following parameters in peripheral blood total number of WBC platelet counts percentage of blast cells or age of the patient

Colony stimulating activity

Sixteen out of 30 patients had CSA in their initial peripheral blood sample (Table I) Peripheral blood cells from these 16 leukemic patients induced an average of 13 8 colonies/10^6 cells (range 1–95) in the over layer of normal cells the corresponding figure being 13 2 when normal feeder cells were used Patient 23 exhibited an extremely high CSA of his peripheral WBC stimulating to 95 colonies/10^6 cells in the over layer from a healthy person Excluding this patient the other 15 had CSA within the normal range (mean 8 3 colonies/10^6 cells) CSA in the peripheral blood of the majority of leukemic patients therefore tends to be slightly lower than in a normal population

There was no statistically significant correlation between CSA and peripheral monocyte counts (7) percentage of blast cells platelet counts total WBC counts or age or the patient

CSA was mainly unaffected during treatment (Table II) However, four patients who initially lacked CSA acquired this ability after treatment These patients appeared to have a tendency towards a more protracted course than those with prolonged lack of CSA though the difference was not significant

Prognostic evaluation

All 11 of those 12 patients entering remission in whom CFC could be determined had CFC before treatment while only 11 out of 18 non responders had CFC ($p<0.02$ χ^2 test) All of the 12 patients entering remission had CSA but only four of the 18 non responders This difference is statistically highly significant ($p<0.001$ χ^2 test) (Tables I and III) Presence of CSA was accordingly associated with a favorable prognosis in 12 out of 16 patients The lack of both CFC and CSA thus indicates a poor prognosis and CSA seems to be the more important prognostic factor

There was no correlation between the actual number of CFC and CSA on the one hand and the duration of complete remission or survival time on the other (Table IV) Some statistical correlation was however found between the mean number of CFC in the initial sample and remission i e patients with remission(s) had a greater number of CFC than non remission patients ($p<0.1$ Student s *t* test Table I)

The four non responders who had both CFC and CSA did not have a longer survival time than non responders lacking CFC and/or CSA None of them exhibited any signs of beginning remission at the time of death nor did their clinical picture or cause of death differentiate them in any way from other patients dying without remission The only statistically significant difference between the initial sample of these four non responders with both CFC and CSA and the responders with the same combination was an increased number of blast cells in the peripheral blood of the former ($p<0.02$ Student s *t* test Table I) The four non remission patients with CFC and CSA were slightly older as a group than the remission patients (61 2±9 1 vs

Table IV *Patients achieving complete remission(s) pretreatment data on CFC and CSA in peripheral WBC (mean no of colonies/10⁶ cells) and clinical course*

n d = not determinable

Pat no	CFC	CSA	Complete remission (d) First	Complete remission (d) Second	Survival after diagnosis (d)
19	1	2	267		430
20	18	6	248	145	626
21	2	4	720	>360	>1 200
22	13	6	213	183	515
23	71	95	133	>240	> 740
24	55	16	>524		> 572
25	2	2	108		217
26	3	2	>114		> 172
27	n d	10	> 82		> 153
28	26	18	56		> 166
29	4	4	>129		> 188
30	49	28	> 30		> 86

50 8±16 3 years) but the difference is not statistically significant

The mean age of the remission patients was 50 8±16 3 years and of the non remission patients 61 6±13 7 years ($p<0\ 1$ Student s t test) The *mean percentage of blast cells in peripheral blood* also correlated to prognosis with 24 0±26 6 for remission patients and 55 2±33 8 for non remission patients ($p<0\ 02$) Neither total WBC counts nor platelet counts correlated to response to treatment

DISCUSSION

The presence of CFC in peripheral blood from patients with acute non lymphoblastic leukemia has been shown in previous studies (15 17 18) as well as in this one Some authors claim that peripheral WBC from patients with AML have a greater than normal colony forming capacity (18) while others report that a large number of leukemic patients lack CFC in the blood (17) In this study most patients (22/30) had CFC before treatment three of whom had an increased number of CFC and 19 a tendency towards a smaller average number than healthy individuals It has been reported (18) that peripheral WBC from patients with high blast cell counts give rise to the greatest numbers of colonies We did not find any significant correlation between CFC in peripheral blood and number of blast cells or any other common hematological parameter such as total WBC or platelet count Nor did we find any relationship between CFC and the age of the patient Some correlation was however detected between the number of CFC in the initial sample and remission induction i e patients responding to therapy tended to have more CFC than non responders

The patients followed during treatment did not generally show any change in the colony forming ability except during severe leukopenia Six out of the 15 patients who were repeatedly investigated after initiation of therapy developed pronounced leukopenia (≤900 WBC/mm³) during treatment with cytotoxic drugs The colony forming ability which had been present before treatment disappeared in all of them Four of these patients later went into remission and the colony forming ability reappeared when WBC counts returned to normal This suggests that the cytotoxic drugs affect the CFC CSA in peripheral WBC was mainly unaffected during the same treatment as well as during drug induced severe leukopenia This implies that CFC are more sensitive to cytotoxic drugs than the cells with CSA In four patients CSA was absent before but present during treatment Although these patients did not enter remission the course in some of them seemed to be more prolonged than in those without detectable CSA during treatment

Monocytes macrophages are ordinarily the main source of CSA (7 9) The presence of CSA in AML is disputed Several investigators (11 18) found no CSA in peripheral blood of patients with active AML disease Our finding of CSA in 16 out of 30 patients with AML *before treatment is at variance* with these results However several other authors have suggested the production of stimulating substances by AML cells (1 10 16) It has been proposed that CSA may be produced by the leukemic cells This property could represent a normal monocyte cell function retained by the leukemic cells and related to their degree of differentiation (8) Recent liquid culture studies have indicated a relationship between inability to differentiate along the monocyte macrophage pathway and a poor prognosis in AML (2) The latter two reports correspond well with our finding that an initial pretreatment presence of CSA being a normal function correlates strongly to a favorable prognosis We also found a strong relationship between absence of CSA and failing response to treatment

Patients over 60 generally have a lower frequency of remission than younger persons (20). In our patients too age was of some prognostic value. However the exact age below 60 yields little prognostic information. Furthermore a substantial number of patients above this age respond to treatment—four out of our 13 patients over 60 entered remission. Age is therefore of little value in judging the prognosis in the individual patient.

The number of blast cells in peripheral blood seems to have some prognostic value in this study, a greater number being associated with a poorer prognosis. However, the variation was so wide spread that the blast cell count is of little value for the individual prognosis.

Our conclusion is that the presence of both CFC and CSA in peripheral WBC is a sign of a good prognosis. Absence of CFC and/or CSA in the initial sample indicates that the patient is unlikely to respond to conventional therapy with cytotoxic drugs. The presence or absence of CSA alone has a higher prognostic significance than the presence or absence of CFC.

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Carcinoembryonic Antigen in Amniotic Fluid

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ABSTRACT Carcinoembryonic antigen (CEA) a substance which is known to occur in high amounts in the fetal gut and also in certain tumors of the gastrointestinal tract has been demonstrated in amniotic fluids from different stages of pregnancy Radioimmunoassays of CEA in amniotic fluids of 91 normal pregnancies showed a decrease from a mean of 53 ng/ml at 19 weeks to 25 ng/ml at the end of gestation The CEA activity in amniotic fluid was eluted in the same volume as a standard ^{125}I CEA on a Sephadex G200 column Amniotic fluid therefore contains CEA similar in molecular weight to the CEA purified from liver metastases of colonic cancer Among 17 cases of abnormal pregnancies CEA elevations were observed in five with anomalous fetuses

Several biological parameters of amniotic fluid have been studied to determine their value in estimating gestational age and in predicting fetal malformations Carcinoembryonic antigen (CEA) originally described by Gold and Freedman (3) is present in high amounts in the fetal entoderm mainly the gut and can be demonstrated in extracts of fetal gut at 2–6 months of gestation Later during development non specific cross reacting antigen (NCA) replaces CEA and is present in the adult colon (6 7) CEA reappears in high amounts in colonic and rectal adenocarcinomas and patients with such tumors usually have increased levels also in their serum (11 12)

The demonstration by Goldenberg et al (5) of high CEA levels in meconium prompted us to determine the normal levels of amniotic CEA and to investigate whether pathological values are present in fetal disorders This paper reports on an evaluation of the levels of CEA in amniotic fluid during normal pregnancy and in some abnormal pregnancies

MATERIAL

Amniotic fluid was obtained from 108 women of whom 17 carried fetuses with various defects (Table I) Chromosome analysis of the amniotic fluid cells and α fetoprotein (AFP) determination were performed in all cases CEA values from cases ending with the birth of an apparently healthy child were used to construct the normal curves Fig 1 gives the mean $\pm$S D for each gestational age

METHODS

CEA was quantified by measuring the inhibition of a monospecific antiserum reacting against ^{125}I-CEA The direct radioimmunoassay (RIA) was performed with the Roche reagents (Hoffmann La Roche Basle) 100 μl of amniotic fluid was mixed with 4 9 ml of 0 1 M ammonium acetate at pH 6 8 and 25 μl of diluted goat anti-CEA antiserum was added The reagents were mixed incubated at 45 C for 30 min and 25 μl (120 000 cpm) of ^{125}I CEA was added After incubation for 30 min 2.5 ml of zirconyl phosphate gel (Z gel) was used to precipitate antigen–antibody complexes The amount of bound ^{125}I CEA in the precipitate was counted CEA concentrations were then determined with the aid of standard inhibition curves

Chromatography of amniotic fluid was performed on a Sephadex G200 1 8×85 cm column (Pharmacia Uppsala) Analysis of 2 ml fractions was made using the direct Z-gel assay

RESULTS

The mean concentration of CEA in the amniotic fluid was 53 ng/ml during the 19th week and then

Table I *CEA in amniotic fluid from abnormal pregnancies*

Case no	Clinical indication/diagnosis	Result	Week of gestation	CEA (ng/ml)	AFP (μg/ml)
1	Previous child with cheilognathopalatoschisis	46 XY born with cheilognathopalatoschisis	18	50	29
2	LSD addict	46,XX twins (I malformed right arm and both feet II normal)	17	26	15
3	Previous child with Mb Down	46 XX t(11 22) (q 23 q 12)	16	44	16
4	Old age	47,XXY induced abortion	17	33	15
5	Previous child with Mb Down	47,XXX induced abortion	18	49	29
6	Previous unbalanced translocation	46 XY sudden death at 4 mo after birth	17	41	*47*
7	Previous child with Mb Down	47,XY +21 induced abortion	18	12	32
8	Chromosomal alterations in the family	47,XXY induced abortion	18	74	20
9	Previous child with 47 +D	46 XY spina bifida induced abortion	16	22	*131*
10	Previous child with malformations	46,XY spina bifida induced abortion	19	*84*	*110*
11	Old age	46 XY anencephaly (died 5 d after birth)	20	22	*545*
12	Polyhydramnios	47 XX +18	33	22	*17*
13	Sampled at delivery	46 XY partial acrania died soon after birth	31	13	*29*
14	Sampled at delivery	46,XX partial acrania died soon after birth	37	*59*	*37*
15	Sampled at delivery	46 XX acrania died soon after birth	38	*71*	*19*
16	Polyhydramnios sampled at delivery	46 XX anencephaly	37	*46*	*15*
17	Sampled at delivery	46 XX acrania dead	36	*58*	*20*

[a] For normal values see Nørgaard Pedersen et al (8)
Figures in italics are values above mean+1 S D for age

declined to a mean of 25 ng/ml at 36 weeks ($p <$ 0 05 *t* test) (Fig 1) The range of values was rather wide as illustrated by the S D Discolored samples indicating the presence of meconium usually had the higher CEA values. Such samples contribute to the variation of CEA values at each gestational age

The CEA activity was demonstrated in the same

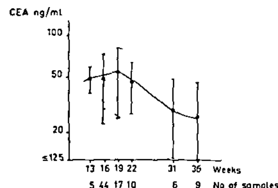


Fig 1 CEA levels (±S D) at various times during normal pregnancies

elution volume as the purified [125]I CEA of Hoffmann La Roche (Fig 2) Amniotic fluid therefore contains CEA similar in molecular weight to the CEA derived from liver metastases of colonic cancer

In addition to the amniotic fluids from pregnancies resulting in normal births samples were available from cases with chromosomal defects or malformations Cases 10 and 14–17 showed raised CEA levels (Table I) The CEA values of abnormal cases did not seem to have the same diagnostic value as AFP determination in cases of neural tube defects (Table I) (9)

DISCUSSION

The presence of CEA in the fetal gut has been detected in early pregnancy but not during the last three months (3) These studies made use of immunodiffusion With a sensitive RIA we found amniotic CEA throughout the pregnancy the concentration decreasing prior to term The primary

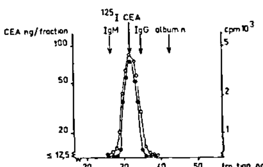


Fig 2 Gel filtrations of amniotic fluid (●—●) from sam ples at 16–36 weeks of gestation on a Sephadex G200 column together with purified ¹²⁵I-CEA (O—O) and the normal serum markers IgG IgM and albumin

source of CEA in the amniotic fluid is probably the fetal gut during the first two trimesters The de crease suggests that there is some metabolic breakdown of CEA during fetal life In animals CEA is metabolized by the liver (10) this may be the case in humans too During a normal pregnancy the mother s serum appears to have a low level of CEA (4) the fetal CEA may therefore accumulate in the fetal liver

Meconium consists largely of glycoproteins (2) One mg of meconium at birth has been shown to contain around 200 ng of CEA (5) Contamination of the amniotic fluid with meconium should therefore involve increased amniotic CEA values We have confirmed an association between discoloration of the sample and high CEA values in amniotic fluid Discoloration by meconium may in turn indicate fetal distress Late in pregnancy amniotic CEA was increased in four of the present cases with severely distressed fetuses This was also the case in one abortion induced on account of high amniotic AFP

There is preliminary evidence (work in progress) that NCA (1 6) is also present in amniotic fluid Unlike CEA the level of cross reacting substances increases during gestation Since CEA decreases while cross reactive substances increase during normal pregnancy the CEA/NCA quotient might be a useful way of determining gestational age However the calculation of such a quotient re quires a weight to-weight comparison which can only be made with RIA standard curves of purified NCA and these are now in progress

There was no obvious relationship between abnormal CEA and raised AFP Amniotic fluids from fetuses which may be expected to have abnormal CEA such as congenital nephrosis with protein leakage or liver disorders with decreased CEA metabolism were not represented in the pres ent series Such cases should be investigated

ACKNOWLEDGEMENT

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Table I *CEA in amniotic fluid from abnormal pregnancies*

Case no	Clinical indication/diagnosis	Result	Week of gestation	CEA (ng/ml)	AFP[a] (μg/ml)
1	Previous child with cheilognathopalatoschisis	46 XY born with cheilognathopalatoschisis	18	50	29
2	LSD addict	46,XX twins (I malformed right arm and both feet II normal)	17	26	15
3	Previous child with Mb Down	46,XX t(11 22) (q 23 q 12)	16	44	16
4	Old age	47 XXY induced abortion	17	33	15
5	Previous child with Mb Down	47 XXX induced abortion	18	49	29
6	Previous unbalanced translocation	46 XY sudden death at 4 mo after birth	17	41	*47*
7	Previous child with Mb Down	47,XY +21 induced abortion	18	12	32
8	Chromosomal alterations in the family	47,XXX induced abortion	18	74	20
9	Previous child with 47 +D	46 XY spina bifida induced abortion	16	22	*131*
10	Previous child with malformations	46 XY spina bifida induced abortion	19	*84*	*110*
11	Old age	46,XY anencephaly (died 5 d after birth)	20	22	*545*
12	Polyhydramnios	47 XX +18	33	22	*17*
13	Sampled at delivery	46 XY partial acrania died soon after birth	31	13	*29*
14	*Sampled at delivery*	*46 XX partial acrania died soon after birth*	*37*	*59*	*37*
15	Sampled at delivery	46,XX acrania died soon after birth	38	*71*	*19*
16	Polyhydramnios sampled at delivery	46 XX anencephaly	37	*46*	*15*
17	Sampled at delivery	46,XX acrania dead	36	*58*	*20*

[a] For normal values see Nørgaard Pedersen et al (8)
Figures in italics are values above mean+1 S D for age

declined to a mean of 25 ng/ml at 36 weeks ($p<0.05$ *t* test) (Fig 1) The range of values was rather wide as illustrated by the S D Discolored samples indicating the presence of meconium usually had the higher CEA values Such samples contribute to the variation of CEA values at each gestational age

The CEA activity was demonstrated in the same elution volume as the purified ^{125}I CEA of Hoffmann La Roche (Fig 2) Amniotic fluid therefore contains CEA similar in molecular weight to the CEA derived from liver metastases of colonic cancer

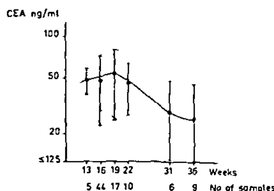


Fig 1 CEA levels (±S D) at various times during normal pregnancies

In addition to the amniotic fluids from pregnancies resulting in normal births samples were available from cases with chromosomal defects or malformations Cases 10 and 14–17 showed raised CEA levels (Table I) The CEA values of abnormal cases did not seem to have the same diagnostic value as AFP determination in cases of neural tube defects (Table I) (9)

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Hereditary Angioneurotic Oedema in Finland

Clinical Immunological and Genealogical Studies

Kyllikki Ohela

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ABSTRACT A total of 7 families with hereditary angioneurotic oedema (HANE) have been found in Finland Six HANE patients have died from laryngeal oedema, 27 patients with diagnosed HANE are alive and 21 members have a haematological abnormality typical of HANE, i e a deficiency of the inhibitor of the activated first component of complement (C1 INH), but no manifest symptoms The largest family has 363 living members 303 of whom were investigated for C1 INH, C4 and C3 Fourteen patients had HANE, 18 relatives were symptomless but had C1 INH deficiency and 3 members of the family had died from laryngeal oedema In two families only one case of HANE was diagnosed, the parents in both cases being symptomless with normal C1 INH levels All patients who had died from laryngeal oedema and 10 of the 27 HANE patients still alive had a typical triad of paroxysmal abdominal pain peripheral oedema and laryngeal oedema Six patients have had abdominal attacks alone three peripheral oedema alone and two only laryngeal oedema The age at onset of symptoms was 1–51 years Three patients who have received continuous methyltestosterone therapy had hardly any symptoms during the treatment Thirteen patients have received tranexamic acid either during an attack or continuously with positive effects in all except two Cinnarizine treatment was beneficial in three out of four cases both when given continuously or during an attack

Hereditary angioneurotic oedema (HANE) is a rare familial disease transmitted as an autosomal dominant trait Affected individuals have recurrent attacks of acute circumscribed oedema involving the skin subcutaneous tissue and the mucous membrane anywhere in the body especially in the pharynx larynx and gastrointestinal tract If the intestinal tract is involved severe abdominal cramp vomiting and diarrhoea occur The mortality rate is high among those affected with acute laryngeal oedema Less frequent symptoms are urine retention due to oedema of the urinary tract and symptoms from the central nervous system such as severe headache aphasia and hemiplegia (4 34)

Osler (34) was the first to describe a family with HANE in 1888 The underlying biochemical defect is a deficiency of the inhibitor of the activated first component of complement (C1 INH) (13) Attacks of HANE are associated with the generation of activated C1 esterase (C1) in the plasma (15) The natural substrates C4 and C2 are consumed so that their plasma concentrations are reduced even during asymptomatic intervals (1 7 15) The split products of C2 are thought to have a kinin like activity and to produce oedema by increasing the permeability of the capillary walls (14 22) The activation mechanism of C1 is unclear but may occur via the fibrinolytic enzyme plasmin kallikrein or by antigen–antibody complexes (12 38) Trauma mental stress excessive fatigue infections and menstruation i e conditions in which increased plasmin activity is found seem occasionally to precipitate the attacks but the precise triggering mechanism is unknown (16 23 26 39) A low C1 INH level has been known to precede the clinical disease by years (1 15) Of all those affected with HANE 15% have normal or elevated amounts of an immunologically crossreactive nonfunctional C1 INH protein (2 20 24 40 41)

HANE patients have been reported from various parts of the world Extensive reviews of HANE have been presented for example by Landerman (23) Donaldson and Rosen (16) Hadjiyannaki and Lachmann (19) and Gigli (18) The present paper reports the findings from the Finnish HANE families

Table I Seven HANE families in Finland

Family	Family members examined	Deaths due to laryngeal oedema	HANE patients	Asymptomatic decreased C1 INH
A	61	2	3	–
B	20	–	4	1
C	303	3	14	18
D	23	–	2	1
E	22	1	2	1
F	5	–	1	–
G	7	–	1	–
Total	441	6	27	21

MATERIAL AND METHODS

Criteria for diagnosis

A patient was considered to suffer from HANE if repeated immunochemical determinations showed decreased C1 INH and C4 values and if at least one of the following three symptoms was present peripheral oedema acute abdominal pain laryngeal oedema In addition one patient with three attacks of laryngeal oedema and repeatedly decreased C1 INH levels but normal C4 levels was classified as having HANE Those of the deceased subjects who had had the complete triad of symptoms were considered HANE cases

Material

The present material was collected in 1969–75 in the dermatological clinics of the university central hospitals other central hospitals and in the Hospital for Allergic Diseases in Helsinki Fresh cases of HANE diagnosed elsewhere have been reported to us after laboratory analyses C1 INH determinations have been made in Finland since 1969 at the Municipal Bacteriological Laboratory and at the Laboratory of Clinical Immunology both in Helsinki

The propositi of three families (A B and C) were diagnosed at the South Saimaa Central Hospital in 1969–73 and of three families (D E the third case turned out to belong to family C) in the Hospital for Allergic Diseases in 1966–71 The propositus of family F was diagnosed at the Department of Dermatology Tampere Central Hospital and of family G at the Department of Medicine Seinajoki Central Hospital both in 1972

Genealogical studies

The families were investigated 4–10 generations back through the parish records The available old case histories were studied Data on families A and B have been published earlier (33) Family C turned out to be the largest and the intention was to include all members of the family in the study The family was traced back for 6 generations to the year 1831 The living members totalled 363 All were sent a questionnaire (see Clinical data) and were asked to visit a laboratory for blood tests (C1 INH C4 C3) Such data were obtained from 321 and blood samples from 303 members of the family A total of 189 members were examined in 1973–74 in the South Saimaa Central Hospital and on two occasions in the Health Centre of Luumaki 145 were males and 158 females aged between 4 days and 73 years (including two newborns) In addition 22 individuals who were married to members of this family were studied An old family tree including only male members was available This enabled the family to be traced back two more generations to the forefathers living on the same farm seven generations earlier in 1642 (13 generations altogether) Seventeen descendants of these remote branches were examined HANE was found in none of these but two of them had allergic oedema Three belonged to the group of 22 individuals who were married to members of the family A total of 57 members of families D–G were examined (Table I) 25 were males and 32 females aged between 3 months and 84 years

Clinical data

The preliminary patient data were collected using a questionnaire on the following items attacks of peripheral oedema abdominal pain laryngeal oedema urinary retention and headache age at onset frequency duration and possible provoking factors (trauma mental stress menstruation pregnancy oral contraceptives) prodromal symptoms therapy given and response to treatment Other diseases recorded were atopic eczema allergic rhinitis asthma urticaria rheumatic diseases and hepatitis

In addition to the somatic investigation skin scratch tests were carried out with several allergens on 7 HANE patients suffering from atopy

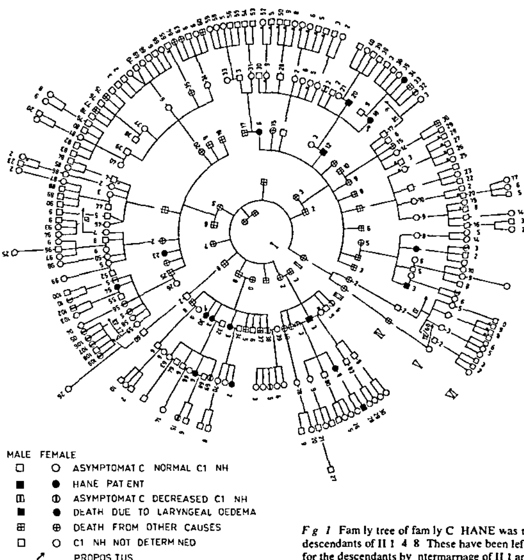
Laboratory analyses

C1 INH C4 and C3 were determined by single radial immunodiffusion (27) in 478 cases The biological activity of C1 INH was determined (25) in 319 cases The analyses were made at the Laboratory of Clinical Immunology in Helsinki Finland Control samples were collected from 244 blood donors The normal range for C1 INH was 60–160% (mean 100 S D 29 3) for C4 0 2–0 65 g/l (mean 0 36 S D 0 11) and for C3 0 55–1 4 g/l (mean 0 96 S D 0 24)

In addition to the routine laboratory analyses the following determinations were made in the 14 in patients thrombocyte count differential leucocyte count GOT GPT alkaline phosphatase bilirubins creatinine CPK Urinary porphyrins were determined in six cases Furthermore in the case of 20 patients and 63 members of family C laboratory data on the following were obtained Latex Waaler Rose cold agglutinins antistreptolysin-O antistaphylolysin direct Coombs cryoprecipitation and antinuclear antibodies (IF) at the Laboratory of Clinical Immunology Helsinki The same tests had been performed previously in 81 members of families A and B (33)

RESULTS

The seven HANE families were found to have 27 affected individuals In addition six have died from laryngeal oedema Asymptomatic members with



F g 1 Fam ly tree of fam ly C HANE was not found in descendants of II 1 4 8 These have been left out except for the descendants by ntermarriage of II 1 and 8

low C1 INH values totalled 21 The distribut on of members and find ngs in the seven families are shown in Table I and the pedigrees in F gs 1 3 except for the information concern ng fam l es A and B wh ch has been publ shed earl er (33) In the largest fam ly C HANE was found in three genera t ons Three have died from laryngeal oedema and 14 l ving HANE patients and 18 asymptomat c members had lo v C1 INH values

Accord ng to the available knowledge the ances ors of fam ly C d d not have symptoms relat ng to HANE A forefather born in 1831 d ed at the age of 90 and h s wife at 59 All of the r n ne ch ldren were free from HANE symptoms accord ng to the data available though the descendants of four of them (C II 2 5 9 12) have HANE The total number of members exam ned and the f nd ngs in various branches of the fam ly are shown in Fig 2

The d sease s severe n two of the four branches and m ld in two All three pat ents who d ed from laryngeal oedema as well as n ne of the 14 HANE pat ents are descendants of C II 2 and 9 who belong to the branch w th severe symptoms

One of the branches w th m ld symptoms has had only two descendants (of C II 5) with symptoms of HANE One of these pat ents was the oldest n th s material a 73 year-old woman (C III 23) who had developed swell ngs and laryngeal oedema only at the age of 49–57 years She has had no descendants The other patient a 45 year-old woman (C IV 54) has had attacks of abdom nal pain only during a one year period at the age of 35 Her father d d not to her knowledge have symptoms of HANE thou

FAMILY C

	II 1	II 2–3	II 4	II 5–6	II 7	II 8	II 9–10	II 11	II 12–13	TOTAL
DESCENDANTS TOTAL	106	96	27	103	-	46	30	-	26	445
DEATHS BEFORE 1974	15	11	6	15	-	2	4		2	55
DEATHS DUE TO LARYNGEAL OEDEMA		2					1			3
LIVING 1974	91	85	21	88		44	26		24	379
EXAMINED	65	65	13	81		42	26		4	316
HANE PATIENTS		5		2			4		3	14
ASYMPTOMATIC DECREASED C1 INH		4		9			4		1	18

DESCENDANTS BY INTERMARRIAGE 16 TRUE NUMBER 429
16 363
13 303

Fig 2 Findings in family C

the number of asymptomatic members with immunochemically low C1 INH values is greatest among his descendants i e 9 asymptomatic members and all except one of these also have low C4 values

In the other branch with mild symptoms (descendants of C II 12) only 3 direct descendants have HANE The age at onset of symptoms was highest 51 years in the grand father (C III 43) he has only subcutaneous swellings His daughter (C IV 81) has only had 5 mild subcutaneous swellings and her 17 year-old son (C V 128) has only had attacks of abdominal pain The oldest asymptomatic member with low C1 INH also belongs to this branch

There are three intermarriages and 16 descendants from these marriages in this family None of the descendants had HANE symptoms and the C1 INH and C4 values were normal in the 13 in whom they could be determined The 22 persons married to members of family C had normal C1 INH and C4 values except one (C IV 6) whose C1 INH was slightly lowered (52% of normal) This person was symptom free and the family comes from the same parish

The home areas of the families are shown in Fig 4 The members are dispersed over southern and middle Finland Some of them have moved abroad

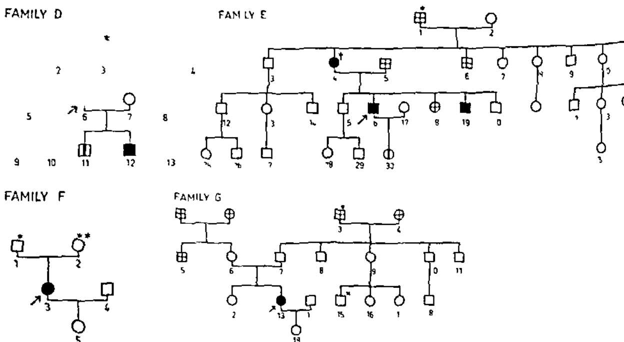


Fig 3 Family trees of families D E F and G Family D traced back to 1776 family E to 1783 family F to 1774 (**to 1839) family G to 1847 Other symbols as in Fig 1

Deaths from HANE

Six patients from three different families have died from laryngeal oedema at ages of 29–49 years (Table II) All of them had a typical triad of symptoms and two (A 25 E 4) had previously been laparatomized because of abdominal pain

In family A the father and grandfather of the propositus had died at the age of 35 and 33 respectively the father from his third attack The father had had his first attack of peripheral oedema at the age of 20 though the abdominal pain had started in childhood The first attack of laryngeal oedema connected with facial swelling had ensued after a visit to the dentist The third fatal oedema had started in the morning and was limited to the throat The patient took a taxi to the hospital 80 km away and died one hour later

Two of the three members of family C were full siblings One of them a 40-year-old man died a few hours after a visit to the dentist the other a 43 year-old woman two days after the onset of facial swelling The third a 49 year-old woman had her first symptoms in her teens and over the course of the years the attacks became more frequent and occurred eventually once a week She had had laryngeal oedema several times since the age of 40

The youngest patient to die from laryngeal oedema was the mother of the propositus in family E She died from her third attack all the attacks had occurred within one year Peripheral oedema had started at the age of 22

HANE patients

Age and sex The age and sex of the 27 HANE patients still alive (17 women aged 14–73 10 men aged 12–69) are shown in Fig 5

Onset and incidence of symptoms The age at onset of symptoms varied from 1 to 51 years (Table III) In eight patients the symptoms appeared before the age of 10 in three of them (D 6 D 12 E 19) at one year In two of these cases the symptoms were subcutaneous swelling and in one attacks of abdominal pain In most patients different symptoms have appeared separately (Fig 6) Most patients had had attacks of abdominal pain as their first symptoms The incidence of various symptoms

Table II *Deaths due to laryngeal oedema*

Family member	Born in	Sex	Age at death (y)
A 12	1893	♂	33
A 25	1924	♂	35
C III 12	1898	♂	40
C III 16	1907	♀	43
C III 31	1911	♀	49
E 4	1922	♀	29

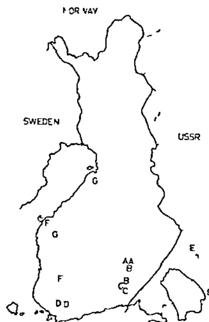
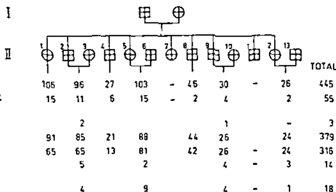


Fig 4 Distribution of the parental birthplaces in seven HANE families

in the 27 HANE patients is shown in Table IV Ten patients have a typical triad

Peripheral oedema has occurred in 19 of these patients and in three of them (C III 43 C IV 72 C IV 81) it has been the only symptom The duration of the attacks of oedema has varied from 1 to 7 days being variable even in one and the same patient on different occasions The most common oedematous areas have been the distal parts of the extremities and the face Swellings have developed also in the genitals and elsewhere in the body and the extremities Seven patients (A 48 C III 23 C III 30 C IV 67 C III 43 F 3 G 13) have occasionally had red stripes and patches at the oedematous sites Two (B 12 F 3) have also had separate red non itching spots of up to palm size and one (C III 30) had faint red circles These signs lasted for a few days

Attacks of abdominal pain were present in 21 cases in six as the only symptom All of these six patients except one are young aged 12–24 years (A 49 A 51 B 19 C V 34 C V 128) The attacks lasted for ½–3 days sometimes a week During the attacks abdominal swelling develops usually associated with vomiting and diarrhoea Laparatomy was performed on seven patients and in surgical reports available a finding of ascites was mentioned



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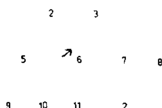
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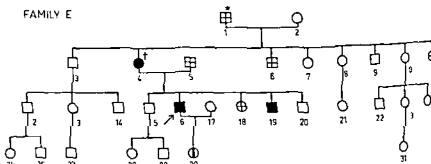
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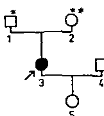
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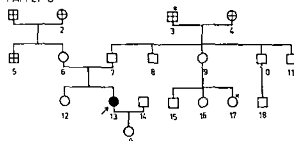


Fig 3 Family trees of families D E F and G *Family D traced back to 1776 family E to 1783 family F to 1774 (**to 1839) family G to 1847 Other symbols as in Fig 1

ly from one to dozens of attacks a year in different patients and even in one and the same patient in different years Only one patient (D 6) has regular weekly attacks Three patients (B 17 B 19 C IV 72) have had only one attack Four of the patients (B 12 D 6 E 19 G 13) have had laryngeal oedemas more than ten times

Prodromal symptoms Seven of the 27 HANE patients had prodromal symptoms prior to the at tack five (A 48 C III 30 C III 33 D 6 F 3) felt tired 1–2 days before the attack In one of these (D 6) the feeling was preceded by a hyperactive period One (E 19) was depressed and nervous and one (C IV 67) had pain in the swollen areas on the preceding day

Triggering factors Most attacks developed with out a clear triggering factor In nine patients though at least some of the attacks were preceded by trauma and/or emotional stress (Table V) The relation to trauma was clearest in three female pa tients (B 12 F 3 G 13) The first attacks developed after severe emotional stress in two patients (C III 16 C III 30)

Four of the 17 female patients often had attacks during menstruation and frequently even in the premenstrual period one also at ovulation (Table V) The symptoms in these cases had also been more frequent during pregnancy One (C IV 7) had the first symptoms during the first trimester of her third pregnancy and another (B 12) having had abdominal pain since childhood had the first pe ripheral oedemas during her first pregnancy

Only four of the female patients had taken oral contraceptives In three of these (A 49 C IV 67 C IV 81) the first symptoms of HANE began when they started to take the pills One of them (C IV 67)

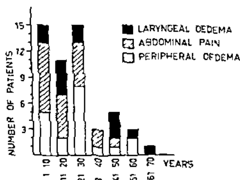


Fig 6 Age at onset of various symptoms in 27 HANE patients

Table IV *Main symptoms in 27 HANE patients*

	No of pats
Abdominal pain	6
Peripheral oedema	3
Laryngeal oedema	2
Abdominal pain + peripheral oedema	5
Peripheral oedema + laryngeal oedema	1
Abdominal pain + peripheral oedema + laryngeal oedema	10

had attacks only during the three years she was taking oral contraceptives (32) In another patient (G 13) they caused severe abdominal pain

Other diseases Seven of the 27 HANE patients had atopy one (D 12) had asthma one (B 12) had asthma and allergic rhinitis and five (A 51 B 17 B 19 B 20 C IV 67) had allergic rhinitis In skin tests they developed allergic reactions to pollens and/or animal epithelia Three patients (B 17 B 19 C IV 67) were given desensitization therapy with good results No effect on the HANE symptoms was observed from this treatment One patient (B 19) has vitiligo and another (C IV 8) migraine The case history of patient C III 33 revealed 7 attacks of pneumonia several attacks of pyelitis as well as tuberculosis in the jugular glands traumatic cere bral concussion and occasional articular pain and elevations of BP Her sister (C III 30) has had goitre and maxillary operation One patient (E 19) has had hepatitis

Of the 303 members examined in family C 25 had atopy and 11 articular symptoms Three had had hepatitis Of recessively hereditary diseases gar goylism occurred in three siblings and Dubin John son syndrome in one member

Results of quantitations The values for C1 INH C4 and C3 in the HANE patients are shown in Table III In four cases the C3 value was above the normal range (1 4 g/l)

In the asymptomatic cases with decreased C1 INH values (Table VI) the mean C4 value (0 16 g/l) was also significantly below normal compared with the normal healthy members (0 42 g/l $t=6\ 69$ $p<0\ 00001$) The mean C3 value (1 12 g/l) was slightly lower than in the HANE patients (1 25 g/l $t=1\ 78$ $p=0\ 08$)

The scatter of the C1 INH and C4 values in f ily C is shown in Fig 7 The C1 INH values

Table V. *Possible triggering factors*
NU = not used

Family member	Sex	Triggering factors: Trauma	Mental stress	Menstruation	Pregnancy	Oral contraceptives
A 48	♂	±	+			
B 12	♀	+	−	+	+	NU
C III 33	♀	−	+	−	−	NU
C IV 7	♀	−	+	+	+	NU
C IV 67	♀	−	−	−	−	+
C V 128	♂	−	+			
D 6	♂	+	+			
E 19	♂	+	+			
F 3	♀	+	−	+	+	NU
G 13	♀	+	+	+	+	+
Total		6	7	4	4	2

members were normal according to immunochemical analysis, but the enzyme was biologically inactive, maybe due to a procedural error during storage and transport of the samples. All these members were asymptomatic. No other immunological deviations were noted compared with the control material.

Occasional leucocytosis was observed in three patients (A 48, B 12, D 6) during the attacks, the counts being as high as 18 500. Other laboratory values were normal.

DISCUSSION

Only nine of the 27 living HANE patients in seven families were diagnosed on the basis of symptoms

Table VI. *Findings in 21 symptom-free members with low C1 INH*
ND = not determined

Family member	Age when examined (y.)	Sex	C1 INH (% of normal): Immunochemical technique	Enzymatic technique	C4 (g/l)	C3 (g/l)
B 18	15	♀	12	ND	0.04	1.32
C III 38	46	♂	47	0	0.09	1.06
C III 46	53	♀	44	0	0.26	1.49
C IV 23	42	♂	35	0	0.18	1.29
C IV 53	45	♂	47	0	0.19	1.11
C IV 56	36	♀	45	20	0.28	1.14
C IV 57	31	♀	30	50	0.15	1.10
C IV 69	30	♀	42	0	0.16	1.09
C IV 75	14	♀	48	0	0.10	1.02
C V 7	19	♀	33	0	0.19	1.00
C V 13	19	♀	35	0	0.20	0.99
C V 32	27	♀	54	30	0.30	0.86
C V 101	19	♀	44	0	0.17	1.46
C V 102	22	♂	29	16	0.09	1.09
C V 105	11	♀	36	0	0.17	1.08
C V 106	10	♀	35	25	0.13	1.11
C V 107	8	♀	37	50	0.13	1.12
C V 109	5	♀	36	75	0.13	1.03
C V 115	4	♀	49	0	0.17	1.2[illegible]
D 11	12	♂	9	ND	0.17	1.05
F 30	6	♀	39	ND	0.14	0.97

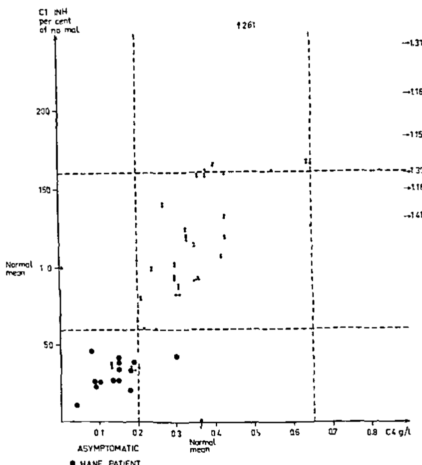


Fig 7 Distribution of C1 INH and C4 values in 14 HANE patients and in 289 asymptomatic family members of family C ≈5 and 95 percentiles for the control series

They all had a typical triad of symptoms The other 18 patients were found during the genealogical studies

Differential diagnosis should cover all allergic and atopic swellings which occur in combination with abdominal pain and laryngeal oedema In those cases urticarial wheals are a common reaction which is not usually characteristic of HANE patients On the other hand HANE patients may have non itching red stripes rings or patches (16 34 46 48) this was the case in nine patients in this material Seven patients had in addition atopy in the form of either allergic rhinitis or asthma which had complicated the diagnosis of HANE

HANE is not usually diagnosed if the patient has attacks of abdominal pain as the only symptom as did six patients is this material The attacks may be attributed to hysteria as was the case in two patients C1 INH can also be low in other states e g haemolytic anaemia the terminal stage of pregnancy (11) infectious hepatitis (43) SLE (42) and lymphosarcoma (6) None of these states was diagnosed in any of the symptom free individuals with a low C1 INH value in this material

The incidence of HANE in Finland is approximately the same as in Sweden where 38 known HANE cases were alive in 1972 (4) Since only three of the nine patients of the present study who were diagnosed on the basis of symptoms turned out to belong to the same family it is obvious that 4–5 times more HANE families than those presented here possibly exist in Finland although not yet discovered

Family C is to our knowledge the largest family studied anywhere The second largest family studied so far had 115 members 36 of whom had HANE and 6 had died from laryngeal oedema (50)

All HANE patients found in Finland have the more common form of HANE with low C1 INH in immunochemical analyses The other form in which C1 INH is normal or elevated when measured immunochemically but is biologically inactive

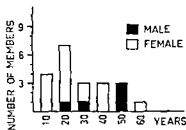


Fig 8 Age and sex distribution of 21 asymptomatic family members with decreased C1 INH

has not yet been found in Finland though the requisite biological determinations have been feasible so far to only a limited extent

HANE is inherited as an autosomal dominant trait with incomplete penetrance Usually the dominance is regular with those affected transmitting the disease to approximately half of their children (23) in a series of 36 families a generation was skipped in only five families in which the disease was apparently passed on by normal parents However isolated cases without family histories have been reported (16 26 49) One instance of HANE occurred de novo in an individual whose parents had normal serum levels of C1 INH (1)

An autosomal dominant inheritance for 2 to 3 generations was found in five of the seven Finnish HANE families (A–E) Only one HANE patient each was diagnosed in two families (F and G) Since both the parents of the propositus and the only child in both families were normal and had normal C1 INH and C4 values and there appeared to be no members with symptoms of HANE blood tests from all members of the family were not considered to be indicated

Family B (33) also differs from the others in that both parents of the propositus were symptom free and had normal C1 INH and C4 values but all four children (daughters) had blood values suggestive of HANE and three also had symptoms of HANE

Skipping of a generation was not verified in patients with HANE symptoms However it is obvious that in family C the disease has skipped one or two generations since according to the hereditary knowledge neither the four siblings whose progeny have HANE nor their parents are known to have had any symptoms connected with HANE In two branches of this family (the descendants of C II 2 and C II 5) the disease may even have skipped a third generation i e the subjects C III 4 and C III 25 However C III 4 often had abdominal pain according to her daughter Skipping of three generations has been reported in patients with HANE symptoms (4)

As for low C1 INH and C4 values no generations were apparently skipped in this material and neither was such a phenomenon found in the literature

The inheritance is not uniform in the different families Some of the families are small and chance may play a considerable role which makes it difficult to draw conclusions In the largest family (C) and possibly also in families A and E HANE is probably caused by the same allele In the other families it may be caused by one or more other alleles In families F and G which have only one HANE patient each and in family B where the blood values of the parents of the propositus are normal it may be a question of novel genetic mutation

In the seven families there were altogether 134 persons who might have HANE Ninety nine are still alive and blood tests have been performed in all but two of them Members with low C1 INH values totalled 48 of whom 27 had clinical symptoms of HANE *The penetrance of the biochemical defect is* thus 100% whereas the clinical penetrance is only half of this (56%) As it is possible that more than one allele is involved penetrance percentages were also calculated specifically for family C the values being 100% and 54% respectively

The gene expression varies both by symptoms and by C1 INH values Nevertheless there are no clear differences between the families In all families the propositus has a typical symptomatic triad while in three families with several patients only some of the typical symptoms are present The severity frequency and onset of symptoms are also variable In only one case (D 6) did the symptoms recur regularly once a week Also all families except F and G have had asymptomatic members whose blood values are typical of HANE

No connection was found between the families Branches of families F and G originate from Vaasa families A and B both from the same parish Juva and family C and some branches of family B from Savitaipale Juva and Savitaipale belong to the isolated areas of Finland where several rare recessive diseases have been found (31) This is possibly due to a primary enrichment of rare genes caused by numerically modest and slow early immigration

and small breeding units which have been typical of the Finnish rural population (29) If negative selec tion is very weak or the disease is manifested late in life evidence of gene enrichment of rare dominant disease might be found (28 29 35)

In the present series there are so far six patients with a lethal outcome giving a total mortality rate of 20% Age at death varied from 29 to 49 years which might well shorten the period of reproduc tion Even a slight negative selection would during some 20–30 generations eliminate the genes intro duced by the founders of the present subpopula tions Thus it seems plausible that the families of the present series have originated from local mutations rather than from enrichment well documented in cases involving the rare recessive genes

The asymptomatic subjects with low C1 INH values formed a special group originally 25 members of five different families In four of them (A 49 A 51 B 19 D 12) the symptoms appeared approximately 5–13 months after the low values had been demonstrated The majority, 18 of the remain ing 21 subjects are members of family C This is the greatest number from one family reported so far (9 16) Half of these 18 subjects belong to one branch of family C (the descendants of C II) which has the smallest number (two) of patients with HANE symptoms In addition families B D and E each have one asymptomatic member with low C1 INH values The age of these subjects varies from 6 to 53 years (Fig 8) Most of them are young but 7 are over 30 years of age All but four also have low C4 values (Table VI)

HANE symptoms may begin at any age from early childhood to late adulthood though most fre quently at ages below 10 years (4 16 23) The latest onset of symptoms reported was at the age of 58 and the patient died at 62 from laryngeal oedema (49) The age at onset of symptoms in this material varied from 1 to 51 years being in half of the cases under 20 and in one third under 10 years In the largest family (C) however the age at onset was remarkably higher in 14 patients only in one case did the symptoms appear before the age of 20 (at 13) and in three cases not until after the age of 45 So it seems likely that at least some of the symptom free members in this family with low C 1 INH values will develop clinical symptoms later in life

The treatment is problematic because corticoids and antihistamines are usually of no help and there is always the threat of laryngeal oedema Specific treatment with C1 INH concentrate is not yet avail able although it has been used in clinical trials (44 51) *The half life of C1 INH is short so continuous* therapy will probably not be possible (5) However in attacks of laryngeal oedema C1 INH con centrate might prove to be a lifesaver Fresh frozen plasma has been given as substitution treatment (21 37) This however is not usually available im mediately and it may cause an anaphylactic reac tion which happened to one patient (E 19) in this material

In some HANE patients attacks have been prevented by continuous treatment with methyl testosterone (47) plasmin inhibitors epsilon amino-caproic acid (EACA) (8 26 30) and tranexamic acid (AMCA) (3 26 45) and cinnarizine (17) the action of the latter being attributed to its blocking effect on C4 (10)

Methyltestosterone was given to three patients (A 48 C IV 7 D 6) and they have been nearly symptom free with a dosage of 12 5–25 mg daily One patient (D 6) has been given this treatment for 9 years with the adverse effect of occasional path ological findings in the liver function tests One patient (A 48) has received the medication for 2.5 years and has developed gynecomastia (33) as a side-effect

Cinnarizine therapy (Marisan® Leo) (20–30 mg daily) seemed to have a beneficial effect on three of the four patients treated The results of treatment in three cases have been published earlier (32) Un fortunately this preparation has been removed from the Finnish market

Most of the patients in this material 13 altogether were treated with AMCA (Cykloka pron® Kabi) Except in two this seemed to have a beneficial effect on HANE attacks The results of treatment in seven patients have been published earlier (32) The majority received the medication only during the attacks 1 5 g×3 daily Side-effects observed were in one case transient elevation of GPT and fatigue and dizziness in another

The patients who have had only a few HANE attacks and the symptom free patients with low C1 INH values have been informed about AMCA medication They have also been advised to take AMCA as a prophylactic if they are to undergo surgery A 17 year old boy (C V 128) who has had abdominal pain as the only symptom had a tonsil lectomy under AMCA protection EACA

laxis during operations has been reported (36) In the event of severe oedema attacks the first aid instruction is AMCA i v followed by methylprednisolone because some of the patients also have allergies The clinic must also be prepared for intubation or tracheostomy if necessary The patients have been given a treatment card which includes diagnosis and treatment instructions to be kept with them always

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ADDENDUM

Since this paper was written two more HANE families have been found in Finland In one of them the affected members have the rarer form of HANE with C1 INH values above the normal level but biologically inactive

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Table I *Data on the patients*

A =effective valve area (cm²) A_i=internal orifice area (cm²) A_o=overall valve area (cm²) $e_i=A_e/A_i$ $\dot{Q}$=cardiac output (l/min) PR=pulse rate R=rhythm, SR=sinus rhythm AF=atrial fibrillation B-S=Björk-Shiley L-K=Lillehei-Kaster

Valve	Pat no	State	$\dot{Q}$	PR	R	Manometry A_e	Ultrasound A	Ultrasound e_i
B-S 29	1	Rest	3 6	74	SR	1 4	2 5	0 54
A_i 4 6		Work	8 4	101	SR	2 0	2 9	0 63
A_o 6 6								
B-S 31	2	Rest	4 4	81	SR	1 4	1 8	0 39
A_i 4 6	3	Rest	7 2	59	SR	1 7	1 7	0 37
A_o 7 5	4	Rest	4 8	56	SR	1 9	1 9	0 41
	5	Rest	4 4	65	AF	1 0	1 8	0 39
L-K 18	6	Rest	3 6	55	AF	1 1	1 4	0 56
A_i 2 5		Work	3 4	91	AF	0 8	0 7	0 28
A_o 5 1	7	Rest	4 4	85	SR	1 7	1 6	0 64
L-K 25	8	Rest	7 7	82	SR	2 8	2 5	0 51
A_i 4 9		Work	9 2	106	SR	2 3	2 3	0 47
A_o 8 3	9	Rest	9 5	67	AF	3 3	3 4	0 69
		Work	10 5	80	AF	3 3	3 5	0 72
Beall medium	10	Rest	4 8	56	AF	1 7	1 7	

in vitro studies of disc valve obstruction and with the findings of other investigators It should be noted that the non invasive determination of mitral flow rate and disc valve insufficiency does not feature in the investigation

MATERIAL

The patient material consisted of 10 adults with mitral disc valve implants Patient 10 was 4 years postoperative the others ½–1 year postoperative The patients were in functional classes I–II and on a maintenance regime of digitalis diuretics and anticoagulants In patients with L-K (Lillehei-Kaster) or B-S (Björk-Shiley) valves the larger valve opening was oriented posteriorly Angiography at rest performed in conjunction with the present investigation disclosed a large disc valve insufficiency in patient 10 whereas the insufficiency was zero or insignificant in the others Further patient data are presented in Table I

METHODS

Ultrasound equipment

The ultrasound equipment consisted of a modified (5) Hewlett Packard Sound Monitor and a Kay Sona-Graph 6061B Sound Spectrum Analyzer The output of the Sound Monitor was recorded on magnetic tape and subsequently frequency analyzed on the Sona-Graph

Data collection

Data were collected with the patient in the supine position on the catheterization table The cardiac output at rest was determined with the direct Fick method during a 3-min period The right heart catheter (Cournand) was then advanced to the pulmonary artery wedge position and the left heart catheter (polyethylene) to the left ventricle With the ultrasound probe on the left anterior chest the region of the mitral valve was scanned manually with the ultrasonic beam and the probe position that resulted in the largest diastolic frequency shifts was determined with the aid of the audio signal of the Sound Monitor With the probe in this position the ultrasound data the pulmonary artery wedge pressure and the left ventricular pressure were recorded simultaneously In 4 patients data were also collected in a similar manner during work at a constant rate while pedalling in the supine position

Definitions

c=velocity of sound in tissues (1 5 10⁵ cm/sec) Δf=frequency shift (Hz) f=frequency of incident ultrasonic beam (2 1 kHz) θ=angle between axis of incident ultrasonic beam and blood velocity vectors ΔP=diastolic pressure gradient across valve (mmHg) $\overline{\sqrt{\Delta P}}$=mean square root of diastolic gradient (mmHg$^{1/2}$) ϱ=mass density of blood (1/981 g-s²/cm⁴) V=diastolic blood velocity in valve (cm/sec) $\bar{V}$=mean diastolic blood velocity (cm/sec) q=mitral flow rate (cm³/sec) Q=mitral flow rate (cm³/min) A =effective valve area (cm²) T_d=diastolic duration (sec/min)

Equations

Doppler equation $V = \frac{c\ \Delta f}{2\ f\ \cos\theta}$ [1] (7)

Torricelli's law $\Delta P = \frac{1}{2}\rho V^2$ [2] (6)

Orifice equation $\Delta P = \left[\frac{\rho}{2.72\ A_e^2}\right] q^2$ [3] (6)

Orifice equation $A = \frac{Q}{V\ T_d}$ [4] (6)

Orifice equation $A = \frac{Q}{51.7\sqrt{\Delta P}\ T_d}$ [5] (6)

The three orifice equations are equivalent and are based upon Torricelli's law and the additional requirement that A_e is constant.

Calculation of A. The stored ultrasound data were frequency analyzed on the Sona-Graph and the time course of the maximum diastolic frequency shift was determined from the hard copy of the analysis. The maximum frequency shift was integrated and the integral divided by the appropriate diastolic duration to obtain the mean maximum diastolic frequency shift. V was then determined from the Doppler equation using $\cos\theta = 1$. T_d was obtained by measuring diastolic and whole beat durations on the hard copy of the frequency analysis and performing the appropriate calculations. A_e was then determined from eq. 4 using the cardiac output as determined by the Fick method as Q.

The time course of ΔP was constructed from the pressure tracings by subtracting the left ventricular pressure from the wedge pressure. Prior to the subtraction a phasic correction of 0.08 sec was applied to the tracings. The time course of $\sqrt{\Delta P}$ was then constructed and $\overline{\sqrt{\Delta P}}$ determined by integration and subsequent division of the integral by the appropriate diastolic duration. T_d was determined from the pressure tracings and A from eq. 5 again using the cardiac output as Q.

Generally 3–4 consecutive beats were used for the above determinations.

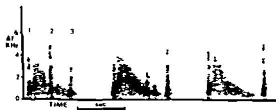


Fig. 1. Frequency analysis from patient 2. Bjork–Shiley 31. Sinus rhythm. Shaded areas represent diastolic blood velocities; curve enveloping shaded areas (not shown) represent maximum blood velocity in valve. Tall vertical lines (labelled) represent disc motion (1 = opening motion, 2 = motion due to onset of atrial contraction, 3 = closing motion due to onset of systole). Note slight variations in disc motion in subsequent diastolic periods. Δf = frequency shift.

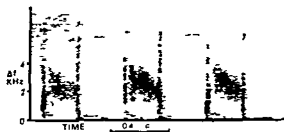


Fig. 2. Frequency analysis from patient 1 resting. Bjork–Shiley 29. Sinus rhythm. Δf = frequency shift.

In vitro tests of disc valves. Tests were performed to determine the in vitro relationship between the pressure gradient and the flow rate in disc valves. The disc valve (L–K 18 and B–S 31) was mounted centrally in an end plate of a plastic tube, i.d. 3.75 cm, length 35.0 cm. The tube was filled with whole blood at room temperature and placed vertically in a constant fluid level reservoir so that the disc valve was 2–3 cm below the fluid surface. The blood was then allowed to flow by force of gravity through the valve and into the reservoir while the pressure inside the tube immediately above the valve was recorded via a pressure transducer recorder system. In this flow situation the pressure gradient across the valve can be calculated from the pressure and the flow rate from the rate of pressure change. Two test runs were performed for each valve tested.

RESULTS

The audio signal of the diastolic frequency shifts from the region of mitral disc valve implants is a soft whispering sound. The apparent optimum probe position was usually identified after 30–60 sec of scanning and was generally located in the 4th or 5th intercostal space 6–8 cm left of the mid sternal line. Frequency analyses of satisfactory quality were obtained in all patients; representative analyses are presented in Figs. 1–2. In these figures the time course of the maximum frequency shift is represented by a curve enveloping the shaded areas. The amount of shading at any particular location in the frequency analysis is related to the energy in the reflected sound. The opening and closing motion of the disc could be discerned in all analyses as heavily shaded vertical lines, the location of which facilitated the measurement of diastolic and whole beat durations. The width of these lines at the base is related to the duration of the opening and closing motion of the disc. At rest these durations ranged from 0.02 to 0.06 sec for the B–S valves and from 0.04 to 0.08 sec for the L–K valves. There was no consistent difference

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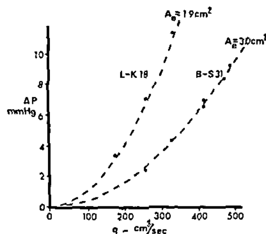


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Another index e_2 defined as the ratio of A_e and overall valve area (A_o), may be useful as an indicator of implant performance as it relates the magnitude of the obstruction to the available tissue area (assuming that available tissue area is identical to overall valve area) Physically e_2 represents the fraction of available tissue area occupied by the blood stream The mean values of e_2 at rest were 0 38 0 24 0 29 and 0 36 for B–S 29 B–S 31 L–K 18 and L–K 25 implants respectively

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Determination of the obstructive qualities of mitral disc valve implants with ultrasound requires that Toricelli s law is valid for the flow in implants and that the position of the ultrasound probe is such that cos θ=1 in the Doppler equation Failure to meet either or both of these requirements will result in overestimation of A_e (6) Toricelli s law is generally valid for the flow in short obstructions when the Reynolds number is sufficiently large The in vitro tests were performed at Reynolds numbers in the range that can be expected in mitral disc valve implants and the results of those tests indicate that Toricelli s law is valid

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Discrepancies in the values of A_e obtained from the ultrasound data vs the manometric data can also reflect errors in the manometric method In the manometric method the left atrial pressure is in essence measured via a compliant catheter consisting of the flow channels between the tip of the right heart catheter and the left atrium Damping of pressure waves in these flow channels can generally be expected to effect overestimation of the gradient and thus underestimation of A_e (eq 5) Patients with powerful atrial contractions can represent exceptions damping of the left atrial pressure rise due to the atrial contraction can result in underestimation of the gradient during the atrial contraction Patient 8 resting is an example of such a phenomenon In this patient the time course of the maximum frequency shift displayed the effects of the atrial contraction distinctly whereas similar effects were absent in the constructed pressure gradient Thus in patient 8 A_e as obtained from the ultrasound data is likely to be the more correct value

Bjork et al (2) and Book (3) studied a group of 24 patients with B–S mitral disc valve implants (18 B–S 29 31 and 6 B–S 27 note that B–S 29 and B–S 31 differ only in the size of the sewing ring) using in part transseptal catheterization to obtain the left atrial pressure The mean valve area in the group as calculated from the Gorlin formula (4) was reported to be 2 6±0 84 cm² at rest and 3 3±0 99 cm² during exercise The Gorlin formula uses a constant of 31 whereas 51 7 is used for the calculation of A_e (eq 5) yielding a tentative correction factor of 0 6 The Gorlin formula also uses the approximation $\sqrt{\overline{\Delta P}}$ (square root of mean gradient) rather than the theoretically correct term $\overline{\sqrt{\Delta P}}$ (6) If it is assumed that the appropriate correction factor for the latter situation is 1/0 87 (see Appendix) the final correction factor becomes 0 69 Thus correction of the mean valve area of 2 6 cm² at rest yields $A_e=1$ 8 cm² which compares favorably with the mean A_e of 1 9 cm² at rest as calculated from the ultrasound data in patients 1–5

For a given valve type (L–K or B–S) the in vitro value of e_1 can be expected to be approximately constant for the various sizes because of geometric similarity The L–K and B–S valves differ somewhat in construction and this probably accounts for the difference in the values of e_1 obtained in the in vitro tests of these two valve types The in vitro tests were performed under rather ideal conditions and it seems reasonable to expect lower values of e_1 in implants than the in vitro values This expectation is supported by the findings of others (2 3) who reported the largest valve area of 3 3+0 99=4 29 cm² Correction of this area with the factor 0 69 allows the calculation of $e_1=0$ 64 which is lower than the value of e_1 found in the in vitro tests of B S 31 In the present investigation the highest values of e_1 were also lower than the corresponding in vitro values Since errors in the ultrasound method will result in overestimation of e_1 these considerations indicate that the values of A_e obtained in implants are reasonable as no gross overestimation of A_e is revealed

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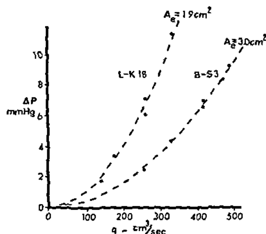


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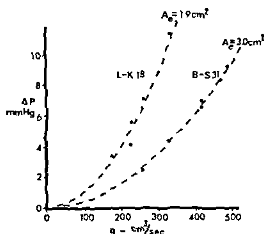


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Ultrasound as a Complementary Diagnostic Method in Deep Vein Thrombosis of the Leg

Rune Lindqvist

From the Medical Department Sodertalje Hospital Sodertalje Sweden

ABSTRACT The ultrasound method for detection of deep vein thrombosis (DVT) of the leg has been used in a study comprising 47 patients This method was compared in the same group with a clinical evaluation and was controlled by venography Clinical evaluation gave a correct diagnosis in 28 (59 6%) of 47 patients, false positive or false negative in 19 (40 4%) With the ultrasound method, the correct diagnosis was made in 41 (87 2%) of 47 patients, false positive in 3 (6 4%) and false negative in 3 (6 4%) No cases were missed of thrombosis situated proximal to the popliteal vein The method was less accurate in cases of thrombosis situated in or distal of the popliteal vein the 3 patients in whom a false negative diagnosis was made belonged to this group Since the ultrasound method has been shown to give few false positive and false negative results, it should be suitable as a screening procedure in patients with symptoms suggestive of DVT

The diagnosis of deep vein thrombosis (DVT) is difficult to establish Hicks (5) among others has shown that only about 1/3 of patients with acute DVT present clinical symptoms In 2/3 of patients developing pulmonary embolism this is the first clinical evidence of thromboembolic disease (1) All the common laboratory methods for the diagnosis of DVT (venography ^{125}I fibrinogen method venous plethysmography) have disadvantages implying that complementary methods should be valuable

In recent years the ultrasound technique at present widely used for foetal monitoring in obstetrics (7) has been applied to a limited extent when diagnosing peripheral arterial disorders (3) and venous disorders in cases of suspected DVT In the latter connection the most important work has been published by Siegel et al (10) who have also employed the method for evaluation of venous insufficiency in the post thrombotic syndrome

When using the ultrasound technique a signal with a frequency of 2–15 mHz is directed via a cutaneous transducer towards the structure to be examined When the signal is reflected against moving structures e g blood corpuscles or heart valves the frequency is altered and on reaching the receiver the shift in frequency is registered as a sonic signal The amplitude of the signal is proportional to the shift in frequency and accordingly yields information about e g the velocity of blood flow in an artery The signal can also be registered and recorded graphically

Harmful effects of ultrasound have been searched for As shown by recent studies (12) exposure times of more than 24 hours can be used without proven risk at a radiation level of 10 mW/cm² which is usually employed in diagnosis of DVT

MATERIAL AND METHODS

The present investigation comprises 47 patients who either attended the Emergency Department Sodertalje Hospital or developed symptoms suggestive of DVT during hospitalization The criterion for entering the study was the presence of distinct X ray findings either indicating or ruling out DVT

The patients were first examined with regard to 13 symptoms related to DVT (Table I) whereafter a preliminary diagnosis was made from the case history and the clinical findings The patients were then examined by the ultrasonic method The clinical examination and the ultrasonic test were both carried out by the same investigator Venography according to the technique of Greitz (4) was performed in all cases after the ultrasound examination and evaluated by the radiologist [illegible] e venography served as the [illegible] s for [illegible] racy of the ultrasound [illegible]

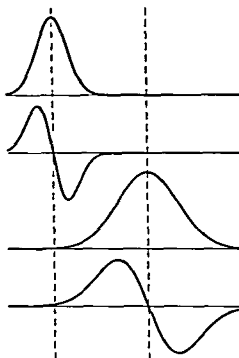


Fig 1 Basis signals used for QRST representation

superior in several respects and is now incorporated into a system which after minor modifications was recently taken into routine use in our Coronary Care Unit (CCU)

TECHNICAL DESCRIPTION

The arrhythmia monitoring system utilizes a minicomputer (Datasaab D5/30) with a core memory of 28 K 16-bit words Eight patients can be monitored simultaneously ECG on/off and alarm reset controls have been added to existing bedside units (Elema Schonander) In routine use a beat by-beat real time analysis of the ECG is performed Before digitalization the ECG signal is passed through an analog low pass filter (f_{3dB}=50 Hz) to eliminate high-frequency artifacts and avoid spectrum aliasing After sampling (100 Hz) the signal is analysed with respect to spikes baseline shifts and powerline noise At a certain level of artifacts the analysis will be blocked for 4 sec Muscle artifacts are suppressed with digital low pass filtering (f_{3dB}=25 Hz) A possible R wave is considered if two first differences with opposite signs and with absolute values above a certain threshold are recorded within 0 25 sec

Program I

This algorithm for VB detection which has been described in more detail elsewhere (6) involves the comparison of all beats with a running average of the complex which dominated at the beginning of the monitoring period (reference complex) If a waveform that has passed the algorithms for detecting R waves and artifacts does not fit properly with the average it is correlated with a typical VB waveform incorporated into the program If the correlation coefficient exceeds 0 8 the complex is marked as ventricular In regular rhythm a 10 % prematurity criterion should also be met before the VB diagnosis is confirmed by the computer

Program II

A waveform recognized as a possible R wave is analysed further and classified as either normal abnormal or artifact This procedure involves the following four steps feature extraction waveform grouping shape classification and final diagnosis

Feature extraction The QRST complex is approximated as a weighted sum of four orthogonal basis functions (Fig 1) The same pairs of functions are used for both the QRS and the ST interval A width parameter has been included for the QRS part of the complex The width parameter of the ST interval as well as the distance between the reference points of the QRS and the ST basis functions are set to fixed standards Consequently five parameters are estimated for each complex the QRS width and the amplitudes of the four basis signals In order to reduce the correlation between the parameters the amplitude coefficients are transformed in pairs into one amplitude and one shape parameter The QRST complex is reconstructed from the above mentioned parameters but only accepted as a true ECG complex if the correlation between the initial and the reconstructed waveform is above a certain threshold

Waveform grouping Complexes with a similar morphology are brought together Each waveform group is characterized by the means and the standard deviations of the five parameters The reference group comprises the waveform that dominates when the monitoring is initiated The grouping of subsequent complexes is based on a distance measure in the five-dimensional parameter space A new waveform group is created when the distance to the existing groups exceeds a fixed limit but this group is considered preliminary until the number of complexes in the group has reached a certain threshold (Fig 2) The parameters of a group are updated whenever a new complex is incorporated A maximum of 10 groups is allowed

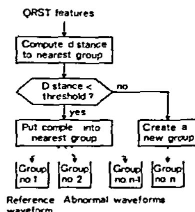


Fig 2 Waveform grouping

for each patient. Adjacent groups are merged at regular intervals and a group without new complexes for a certain period is deleted.

Shape classification. The waveform of the reference group is considered normal. Abnormal waveform groups are separated into four categories: probable VBs, possible VBs, abnormal non-VBs and essentially normal complexes. This classification is based on three linear discriminant functions operating on the parameters of the current waveform (Fig. 3). The coefficients of these functions were determined to minimize the probability of misclassification in a large material of different waveforms recorded from CCU patients and manually classified by two physicians.

Final diagnosis. In this step rhythm data such as prematurity or compensatory pause are combined with the shape classification. Consequently this step must await the recognition of the subsequent QRS complex. The possible transitions from the waveform type to the final diagnosis are shown in Fig. 4. In the present study only the PVBs—below named VBs—were reported by the computer.

Power spectrum analysis

In program II a special subroutine is entered when no normal or supraventricular beats have been detected for 5 sec in a noise free signal. Asystole is diagnosed if no R waves are detected and the power of the signal is sufficiently low.

If these two criteria are not fulfilled the power spectrum of the ECG will be computed. Ventricular tachycardia (VT) is considered when the power spectrum shows a narrow peak within 2–4 Hz. This diagnosis also follows the detection of more than three VBs in sequence with an average frequency above 2 Hz. If the peak in the power spectrum is less marked or above 4 Hz the computer will diagnose ventricular fibrillation (VF). Other conditions will result in an attention alarm if no new complexes are identified within 15 sec.

Artifact alarms

In the present investigation it was decided to study only the arrhythmia alarms given for ventricular arrhythmias.

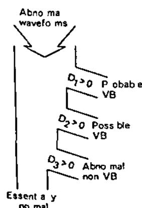


Fig. 3. Preliminary shape classification.

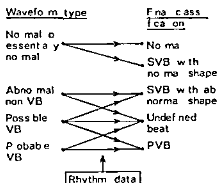


Fig. 4. Final classification of abnormal beats. SVB, supraventricular beat.

These alarm conditions are listed in the method section. Program II also presented four artifact alarms which were defined as follows: 1) check ECG signal: no ECG complexes detected for 15 sec and VT or VF not diagnosed or more than 50% of the preceding minute blocked due to artifacts. 2) VB detection blocked due to artifacts: more than 25% of the preceding minute rejected. 3) low amplitude: VB detection is prevented if the amplitude of the reference complex goes below 0.6 mV. 4) faulty electrode contact: 50 Hz noise in the ECG. At any of the first three artifact alarms mentioned above all abnormal waveform groups were deleted. Artifact alarms were also given by program I but were not studied in detail.

MATERIAL AND METHODS

Patients admitted to the CCU with sudden onset of chest pain less than six hours before admission and with ST-T changes in the resting 12 lead ECG were selected. The general condition of the patients did not influence their selection. A single lead ECG, usually with the electrodes placed over the sternum, was recorded via the conventional monitoring system. A modified Philips 7-channel FM tape recorder was used and recording time was 12 h except in patient 1 who died after 10 h. The study did not interfere with routine ECG monitoring. No criteria were set on the configuration of the ECG complexes or the signal-to-noise ratio. Consequently, no exchange or repositioning of the electrodes was made due to the present study. In most cases the CCU staff was unaware of the recording of the ECG signal.

The attenuation of the ECG signal reproduced from the tape recorder was adjusted at the start of the analysis to obtain a QRS amplitude corresponding to 1.2 mV at the electrodes.

The diagnosis of AMI was based on at least two of the following criteria: 1) acute onset of chest pain with a duration of more than 15 min. 2) typical serial changes in serum CPK values and aminotransferases (ASAT and ALAT). 3) typical ECG changes in the 12 lead ECG. Sixteen out of 23 patients recorded proved to have AMI. However, one recording with peaked P waves and T waves higher than the QRS amplitude was not accepted by

the computer One tape was lost due to a technical mistake One recording was omitted on account of poor technical quality which made a proper interpretation by the physician impossible One patient was excluded because of the occurrence of an interfering ventricular rhythm lasting for about 5 h Thus 12 patients remained for the study QRS interval was calculated as the mean of 5 normal complexes from a 50 mm/sec recording made at the start of the recording Bundle branch block (BBB) was diagnosed from a 12 lead ECG taken on admission The P T and QRS amplitudes were estimated hourly from five representative normal ECG complexes The total number of complexes in each patient was estimated from the average heart rate computed each minute

Tape recorded ECG data were transferred to paper chart (speed 12 5 mm/sec) and analysed beat by beat by a physician Abnormal waveforms were divided into three categories VB=ventricular ectopic beat N=abnormal non ventricular beat X=artifact The diagnosis of a VB was based on subjective criteria using prematurity QRST aberration and increase in QRS width A deviation from normal in at least two of these respects was set up as a requirement for the diagnosis of a VB However narrow premature and slightly aberrant beats were usually classified as N In the event of an uncertain interpretation an abnormal complex was classified as a VB When possible P wave information was used in the classification of a complex Widened beats with abnormal morphology and unchanged P Q interval (intermittent BBB) were classified as N This group comprised abnormal beats not fulfilling the VB criteria Premature complexes with otherwise unchanged morphology were not considered in this study Abnormal beats disturbed by artifacts were classified as VB or N whereas deranged ordinary complexes were classified as X In patients with episodes of ventricular rhythm or VT only 4 ventricular complexes of each episode were counted Also in a run of N beats a max of 4 were added to the total number of non ventricular abnormal beats

The physician s interpretation was considered correct and was followed by a beat by beat comparison with the results from the computer analyses The reasons for false negative and false positive VB detection were analysed with both algorithms for VB detection

A few definitely abnormal complexes overlooked by the physician were detected in the computer analysis When the recording contained several beats of the same configuration the typing of a complex was allowed in retrospect otherwise the complex was considered non ventricular in all cases

The accuracy of the alarms given for ventricular arrhythmias was evaluated by scrutinizing the ECG and the alarm print-out minute by minute False positive alarms due to false positive VB detection were divided into two groups totally irrelevant alarms and alarms with higher priority than the true event False negative VB detection resulted in either the absence of an alarm or an alarm with a lower priority than the real event Ranked after falling priority the alarm situations studied were a) VF and VT (VT=more than 3 VBs with an average frequency above 2 Hz) b_1) more than 3 consecutive VBs b_2) 3 VBs in sequence b_3) paired VBs c) ventricular bigeminy (3 VBs alternating with normal complexes) or more than 5 VBs/min VF and VT will be reported together since program I did not distinguish between these conditions All a or b alarms whether false or missed were taken into account As to the c alarms the following rules were practised 1) An alarm was considered correct if it followed the detection of the first 6 VBs during a running minute 2) If the event was not detected one missed alarm was counted each minute this condition persisted 3) The alarm was not considered if the preceding minute contained an alarm of higher priority 4) After the correct detection of the alarm it was not studied for the next 5 min An a or b alarm was considered correct if printed out by the computer within 15 sec after the start of the arrhythmia even though one or more of the complexes were missed in a run of VBs In the beat-by beat analysis the first 4 complexes were studied in ventricular rhythm or VT For the correct identification of a b alarm the computer had to report whether 2 3 or more than 3 VBs occurred in sequence

The artifact alarms given by program II were counted and the total time during which the arrhythmia monitoring and the VB detection were blocked was computed and presented at the end of the analysis in each patient Also the total period of irregular rhythm—defined as a relative standard deviation of R R intervals (normal beats) above 10%—was computed and displayed after the monitoring period

The analysis could be restarted manually during the 12 hour period This procedure which implied that a new reference complex was computed and abnormal waveform groups deleted (program II) was usually necessary when there was a sudden change in the configuration of the ECG complex Restarting was also necessary in some patients when minor artifacts had deranged the reference complex resulting in false positive VBs (program I) Any missed or false positive VBs recorded prior to the restarting procedure were naturally included in the results The ECG was not studied during resuscitation in two patients

Paired t test on intraindividual numbers of false positive and negative VBs and alarms was used to evaluate any statistical difference in performance between the two computer programs

RESULTS

Some characteristics of the ECG material are presented in Table I Time from onset of symptoms to start of recording varied between 2 and 8 h The total time recorded was 146 2 h or a little more than 12 h per patient The total number of beats recorded was above 600000 QRS interval varied between 60 and 120 msec Permanent BBB was not seen QRS amplitude showed some variation and was usually above the initial value As to the amplitudes of the ECG the quotients P/QRS and T/QRS varied but never exceeded 0 2 and 0 6 respectively

Sinus rhythm dominated in all patients In patient 1 atrial fibrillation prevailed during the first 2 3 h In

Table I *Some characteristics of the ECG material*

Pat no	Onset of symptoms – start of recording (h)	Recorded time (h)	Total no of beats (×10 ³)	QRS duration (msec)	QRS$_{ampl}$ (initial value =1 0) Min	Max	P mpl / QRS$_{ampl}$ Min	Max	T mpl / QRS$_{ampl}$ Min	Max	Irregular rhythm (h)
1	3	10 0	52 5	100	0 7	1 4	0 0	0 1	0 2	0 3	2 6
2	3	12 9	61 9	80	0 8	1 3	0 1	0 1	0 2	0 5	3 9
3	8	11 9	52 5	80	1 0	1 9	0 0	0 1	0 1	0 2	0 1
4	6	12 1	61 1	120	0 6	1 0	0 0	0 1	0 1	0 3	0 5
5	4	12 7	44 5	90	0 7	1 1	0 0	0 1	0 0	0 2	0 5
6	8	12 7	67 4	90	1 0	1 3	0 0	0 1	0 1	0 2	2 9
7	2	12 7	45 0	90	0 8	1 9	0 1	0 1	0 2	0 4	1 2
8	3	12 8	60 9	90	1 0	2 4	0 1	0 2	0 3	0 6	0 5
9	5	12 0	46 8	110	1 0	1 9	0 1	0 2	0 3	0 6	0 1
10	6	12 2	44 1	80	1 0	1 2	0 2	0 2	0 1	0 3	0 1
11	2	12 3	50 0	80	1 0	1 3	0 1	0 1	0 1	0 6	0 5
12	5	11 9	44 5	100	1 0	1 4	0 1	0 1	0 2	0 3	0 1
Total		146 2	631 2								13 0
Mean	4 6	12 2		93	0 9	1 5	0 1	0 1	0 2	0 4	1 1

patients 2 5 6 and 7 irregular rhythm lasted for 0 5–3 9 h and was mainly caused by a sinus arrhythmia In patients 4 8 and 11 premature beats with unchanged morphology resulted in irregular rhythm for about 0 5 h in each Only brief periods of irregular rhythm were seen in patients 3 9 10 and 12

In the beat by beat evaluation (Table II) correct VB identification with programs I and II ranged from 0 to 85% and from 35 to 96% respectively Out of the total number of VBs 2775 the programs correctly diagnosed 48 and 60% respectively this difference was statistically significant ($p<0$ 05) With program II and patient 8 excluded (due to periods with a high frequency of VBs and artifacts) the percentage of correctly identified VBs in pa

Table II *Comparison between ECG classification b) the ph) sician (P) and the computer (C)*
I=program I II=program II

P	VB					Abnormal non VB			Normal		Artifact	
C		VB					VB		VB		VB	
Pat no		I	(%)	II	(%)		I	II	I	II	I	II
1	190	99	52	156	82	38	0	4	2	9	127	15
2	266	74	28	189	71	14	0	1	0	10	2	3
3	15	0	0	8	53	1	0	0	0	0	2	1
4	308	224	73	275	89	574	7	21	11	6	15	19
5	67	4	6	34	51	9	0	1	0	0	1	2
6	54	21	39	19	35	51	0	0	4	23	11	4
7	350	298	85	292	83	11	0	1	4	6	17	7
8	1 321	503	38	559	42	393	9	16	16	20	703	161
9	26	11	42	12	46	0	0	0	341	5	42	11
10	5	3	60	2	40	0	0	0	1	35	17	17
11	61	38	62	25	41	13	0	3	0	13	5	1
12	112	49	44	107	96	0	0	0	14	2	0	0
Total	2 775	1 324		1 678		1 104	16	47	393	129	942	241
Mean	231		44		61							
% of total no of beats	0 44					0 17	0 00	0 01	0 06	0 02	0 15	0 04

Tabl I *Incidence of clinical symptoms of DVT*

	Positive venography		Negative venography but DVT suspected	
	n	%	*n*	%
Swelling (difference in circumference [illegible])	19	79	16	70
Spontaneous p[illegible]	18	75	20	87
Increased c[illegible]	18	75	11	48
Pitt[illegible]	15	63	15	65
D[illegible]	14	58	17	74
Ver[illegible]	8	33	7	30
[illegible]	8	33	4	17
[illegible]	8	33	4	17
[illegible]	7	29	6	26
[illegible]	7	29	9	39
[illegible]	6	25	7	30
[illegible]	6	25	10	43
[illegible]	5	21	3	13
[illegible]	24		23	
[illegible]	6 3	(S D 2 1)	5 7	(S D 2 2)

[illegible] sonicaid [illegible] ny) with [illegible] m² The [illegible]

[illegible] er was [illegible] easily [illegible] difficult [illegible] medially the p[illegible] the femoral vein could [illegible] spontaneous flow rising and falling with the respiration was often heard It was easily distinguishable from the arterial flow which was usually heard simultaneously Compression of the musculature of the thigh with the hand induced a sudden increase in the velocity of the blood flow in the vein which facilitated the examination Diagnosis of DVT was made if no sound signals or a greatly impaired flow over one leg compared with the other were noticed during local compression of the muscles The patient was subsequently examined in the prone position over the popliteal vein The site with the best arterial flow sound was located The venous flow was most often heard at the same site In the event that a small thrombosis is present in one of the deep calf veins blood flow might still be adequate via the other veins In such cases a normal resting flow sound signal will be heard over the popliteal vein With the transducer over the popliteal vein the muscles of the calf were successively compressed more distally The level at which the most distal compression induced a sonic flow signal over the popliteal vein was noted DVT was considered to be present if a difference of more than 5 cm in this level was recorded between the legs Flow signals can rarely be heard over the posterial tibial vein The absence of sonic flow signals from the posterial tibial vein was not considered to be suggestive of DVT in the present study

RESULTS

A total of 47 patients were investigated Venography showed DVT in 24 patients (51 1%) and was negative in 23 (48 9%) Among the positive DVT patients 15 (31 9%) had high thrombosis (proximal to the popliteal vein) and 9 (19 2%) low thrombosis (in or distal of the popliteal vein)

None of the 13 clinical symptoms considered were found to be significantly more common (χ^2 test $p>0\ 01$) in patients with venographically verified DVT than in patients with negative venography The number of symptoms in both groups was essentially the same (Table I) Hohman s sign one classic symptom in DVT tended to be more common in the negative group but the difference did not reach a statistically significant level (χ^2 test $p>0\ 01$)

In an attempt to differentiate DVT clinically from other causes of the symptoms a false positive diagnosis was made in 16 patients (34%) false negative in only 3 (6 4%) thus leaving a number of 28 patients (59 6%) correctly diagnosed

By means of the ultrasound method a correct diagnosis as to presence or absence of DVT was

and small breeding units which have been typical of the Finnish rural population (29) If negative selec tion is very weak or the disease is manifested late in life evidence of gene enrichment of rare dominant disease might be found (28 29 35)

In the present series there are so far six patients with a lethal outcome giving a total mortality rate of 20% Age at death varied from 29 to 49 years which might well shorten the period of reproduc tion Even a slight negative selection would during some 20–30 generations eliminate the genes intro- duced by the founders of the present subpopula tions Thus it seems plausible that the families of the present series have originated from local mutations rather than from enrichment well documented in cases involving the rare recessive genes

The asymptomatic subjects with low C1 INH values formed a special group originally 25 members of five different families In four of them (A 49 A 51 B 19 D 12) the symptoms appeared approximately 5–13 months after the low values had been demonstrated The majority 18 of the remain ing 21 subjects are members of family C This is the greatest number from one family reported so far (9 16) Half of these 18 subjects belong to one branch of family C (the descendants of C II) which has the smallest number (two) of patients with HANE symptoms In addition families B D and E each have one asymptomatic member with low C1 INH values The age of these subjects varies from 6 to 53 years (Fig 8) Most of them are young but 7 are over 30 years of age All but four also have low C4 values (Table VI)

HANE symptoms may begin at any age from early childhood to late adulthood though most fre quently at ages below 10 years (4 16 23) The latest onset of symptoms reported was at the age of 58 and the patient died at 62 from laryngeal oedema (49) The age at onset of symptoms in this material varied from 1 to 51 years being in half of the cases under 20 and in one third under 10 years In the largest family (C) however the age at onset was remarkably higher in 14 patients only in one case did the symptoms appear before the age of 20 (at 13) and in three cases not until after the age of 45 So it seems likely that at least some of the symptom free members in this family with low C1 INH values will develop clinical symptoms later in life

The treatment is problematic because corticoids and antihistamines are usually of no help and there is always the threat of laryngeal oedema Specific treatment with C1 INH concentrate is not yet avail able although it has been used in clinical trials (44 51) The half life of C1 INH is short so continuous therapy will probably not be possible (5) However in attacks of laryngeal oedema C1 INH con centrate might prove to be a lifesaver Fresh frozen plasma has been given as substitution treatment (21 37) This however is not usually available im mediately and it may cause an anaphylactic reac tion which happened to one patient (E 19) in this material

In some HANE patients attacks have been prevented by continuous treatment with methyl testosterone (47) plasmin inhibitors epsilon amino caproic acid (EACA) (8 26 30) and tranexamic acid (AMCA) (3 26 45) and cinnarizine (17) the action of the latter being attributed to its blocking effect on C4 (10)

Methyltestosterone was given to three patients (A 48 C IV 7 D 6) and they have been nearly symptom free with a dosage of 12 5–25 mg daily One patient (D 6) has been given this treatment for 9 years with the adverse effect of occasional path ological findings in the liver function tests One patient (A 48) has received the medication for 2 5 years and has developed gynecomastia (33) as a side effect

Cinnarizine therapy (Marisan® Leo) (20–30 mg daily) seemed to have a beneficial effect on three of the four patients treated The results of treatment in three cases have been published earlier (32) Un- fortunately this preparation has been removed from the Finnish market

Most of the patients in this material 13 altogether were treated with AMCA (Cyklokapron® Kabi) Except in two this seemed to have a beneficial effect on HANE attacks The results of treatment in seven patients have been published earlier (32) The majority received the medication only during the attacks 1 5 g×3 daily Side-effects observed were in one case transient elevation of GPT and fatigue and dizziness in another

The patients who have had only a few HANE attacks and the symptom free patients with low C1 INH values have been informed about AMCA medication They have also been advised to take AMCA as a prophylactic if they are to undergo surgery A 17 year-old boy (C V 128) who has had abdominal pain as the only symptom had a tonsillectomy under AMCA protection

laxis during operations has been reported (36) In the event of severe oedema attacks the first aid instruction is AMCA i v followed by methylpred nisolone because some of the patients also have al lergies The clinic must also be prepared for intuba tion or tracheostomy if necessary The patients have been given a treatment card which includes diagnosis and treatment instructions to be kept with them always

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ADDENDUM

Since this paper was written two more HANE families have been found in Finland In one of them the affected members have the rarer form of HANE with C1 INH values above the normal level but biologically inactive

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Table I *Data on the patients*

A =effective valve area (cm²) A_i=internal orifice area (cm²) A_o=overall valve area (cm²) e_i=A_e/A_i $\dot{Q}$=cardiac output (l/min) PR=pulse rate R=rhythm SR=sinus rhythm AF=atrial fibrillation B-S=Bjork-Shiley L-K=Lillehei-Kaster

Valve	Pat no	State	$\dot{Q}$	PR	R	Manometry A_e	Ultrasound A_e	Ultrasound e_i
B-S 29	1	Rest	3 6	74	SR	1 4	2 5	0 54
A_i 4 6		Work	8 4	101	SR	2 0	2 9	0 63
A_o 6 6								
B-S 31	2	Rest	4 4	81	SR	1 4	1 8	0 39
A_i 4 6	3	Rest	7 2	59	SR	1 7	1 7	0 37
A_o 7 5	4	Rest	4 8	56	SR	1 9	1 9	0 41
	5	Rest	4 4	65	AF	1 0	1 8	0 39
L-K 18	6	Rest	3 6	55	AF	1 1	1 4	0 56
A_i 2 5		Work	3 4	91	AF	0 8	0 7	0 28
A 5 1	7	Rest	4 4	85	SR	1 7	1 6	0 64
L-K 25	8	Rest	7 7	82	SR	2 8	2 5	0 51
A_i 4 9		Work	9 2	106	SR	2 3	2 3	0 47
A_o 8 3	9	Rest	9 5	67	AF	3 3	3 4	0 69
		Work	10 5	80	AF	3 3	3 5	0 72
Beall medium	10	Rest	4 8	56	AF	1 7	1 7	

in vitro studies of disc valve obstruction and with the findings of other investigators It should be noted that the non invasive determination of mitral flow rate and disc valve insufficiency does not feature in the investigation

MATERIAL

The patient material consisted of 10 adults with mitral disc valve implants Patient 10 was 4 years postoperative the others ½-1 year postoperative The patients were in functional classes I-II and on a maintenance regime of digitalis diuretics and anticoagulants In patients with L-K (Lillehei-Kaster) or B-S (Bjork-Shiley) valves the larger valve opening was oriented posteriorly Angiography at rest performed in conjunction with the present investigation disclosed a large disc valve insufficiency in patient 10 whereas the insufficiency was zero or insignificant in the others Further patient data are presented in Table I

METHODS

Ultrasound equipment

The ultrasound equipment consisted of a modified (5) Hewlett Packard Sound Monitor and a Kay Sona-Graph 6061B Sound Spectrum Analyzer The output of the Sound Monitor was recorded on magnetic tape and subsequently frequency analyzed on the Sona-Graph

Data collection

Data were collected with the patient in the supine position on the catheterization table The cardiac output at rest was determined with the direct Fick method during a 3 min period The right heart catheter (Cournand) was then advanced to the pulmonary artery wedge position and the left heart catheter (polyethylene) to the left ventricle With the ultrasound probe on the left anterior chest the region of the mitral valve was scanned manually with the ultrasonic beam and the probe position that resulted in the largest diastolic frequency shifts was determined with the aid of the audio signal of the Sound Monitor With the probe in this position the ultrasound data the pulmonary artery wedge pressure and the left ventricular pressure were recorded simultaneously In 4 patients data were also collected in a similar manner during work at a constant rate while pedalling in the supine position

Definitions

c=velocity of sound in tissues (1 5 10^5 cm/sec) Δf=frequency shift (Hz) f=frequency of incident ultrasonic beam (2 1 kHz) θ=angle between axis of incident ultrasonic beam and blood velocity vectors ΔP=diastolic pressure gradient across valve (mmHg) $\overline{\sqrt{\Delta P}}$=mean square root of diastolic gradient (mmHg$^{1/2}$) ϱ=mass density of blood (1/981 g-s²/cm⁴) V=diastolic blood velocity in valve (cm/sec) $\bar{V}$=mean diastolic blood velocity (cm/sec) q=mitral flow rate (cm³/sec) Q=mitral flow rate (cm³/min) A_e=effective valve area (cm²) T_d=diastolic duration (sec/min)

Equations

Doppler equation $V = \frac{c \cdot \Delta f}{2 \cdot f \cdot \cos\theta}$ [1] (7)

Torricelli's law $\Delta P = \frac{1}{2}\rho V^2$ [2] (6)

Orifice equation $\Delta P = \left[\frac{\rho}{2.72 \cdot A^2}\right] q^2$ [3] (6)

Orifice equation $A = \frac{Q}{V \cdot T_d}$ [4] (6)

Orifice equation $A = \frac{Q}{51.7\sqrt{\Delta P} \cdot T_d}$ [5] (6)

The three orifice equations are equivalent and are based upon Torricelli's law and the additional requirement that A is constant.

Calculation of A. The stored ultrasound data were frequency analyzed on the Sona-Graph and the time course of the maximum diastolic frequency shift was determined from the hard copy of the analysis. The maximum frequency shift was integrated and the integral divided by the appropriate diastolic duration to obtain the mean maximum diastolic frequency shift. V was then determined from the Doppler equation using $\cos\theta = 1$. T_d was obtained by measuring diastolic and whole beat durations on the hard copy of the frequency analysis and performing the appropriate calculations. A was then determined from eq. 4 using the cardiac output as determined by the Fick method as Q.

The time course of ΔP was constructed from the pressure tracings by subtracting the left ventricular pressure from the wedge pressure. Prior to the subtraction a phase correction of 0.08 sec was applied to the tracings. The time course of $\sqrt{\Delta P}$ was then constructed and $\sqrt{\Delta P}$ determined by integration and subsequent division of the integral by the appropriate diastolic duration. T_d was determined from the pressure tracings and A from eq. 5 again using the cardiac output as Q.

Generally 3–4 consecutive beats were used for the above determinations.

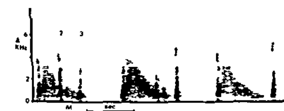


Fig. 1. Frequency analysis from patient 2. Bjork–Shiley 31. Sinus rhythm. Shaded areas represent diastolic blood velocities; curve enveloping shaded areas (not shown) represent maximum blood velocity in valve. Tall vertical lines (labelled) represent disc motion (1 = opening motion, 2 = motion due to onset of atrial contraction, 3 = closing motion due to onset of systole). Note slight variations in disc motion in subsequent diastolic periods. Δf = frequency shift.

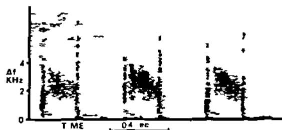


Fig. 2. Frequency analysis from patient 1 resting. Bjork–Shiley 29. Sinus rhythm. Δf = frequency shift.

In vitro tests of disc valves. Tests were performed to determine the in vitro relationship between the pressure gradient and the flow rate in disc valves. The disc valve (L–K 18 and B–S 31) was mounted centrally in an end plate of a plastic tube, i.d. 3.75 cm, length 35.0 cm. The tube was filled with whole blood at room temperature and placed vertically in a constant fluid level reservoir so that the disc valve was 2.3 cm below the fluid surface. The blood was then allowed to flow by force of gravity through the valve and into the reservoir while the pressure inside the tube immediately above the valve was recorded via a pressure transducer recorder system. In this flow situation the pressure gradient across the valve can be calculated from the pressure and the flow rate from the rate of pressure change. Two test runs were performed for each valve tested.

RESULTS

The audio signal of the diastolic frequency shift from the region of mitral disc valve implants is a soft whispering sound. The apparent optimum probe position was usually identified after 30–60 sec of scanning and was generally located in the 4th or 5th intercostal space 6–8 cm left of the midsternal line. Frequency analyses of satisfactory quality were obtained in all patients; representative analyses are presented in Figs 1–7. In these figures the time course of the maximum frequency shift is represented by a curve enveloping the shaded areas. The amount of shading at any particular location in the frequency analysis is related to the energy in the reflected sound. The opening and closing motion of the disc could be discerned in all analyses as heavily shaded vertical lines, the location of which facilitated the measurement of diastolic and whole beat durations. The width of these lines at the base is related to the duration of the opening and closing motion of the disc. At rest these durations ranged from 0.02 to 0.06 sec for the B–S valves and from 0.04 to 0.08 sec for the L–K valves. There was no consistent difference betw

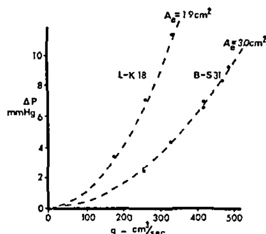


Fig 3 Results of in vitro tests of Lillehei–Kaster 18 (overall valve area 5 1 cm²) and Bjork–Shiley 31 (overall valve area 7 5 cm²) valves demonstrating quadratic relationship between pressure gradient (ΔP) and flow rate (q) ---=plots of eq 3

opening and closing durations The height of the vertical lines is not related to disc velocity because the powerful reflections from the disc surface introduce non linearities in the electronic components of the ultrasound system

In 10 of the 14 determinations A_e as obtained from the ultrasound data was within 0 3 cm² of the value determined from the manometric data (Table I) In the 4 determinations where the discrepancies were larger than 0 3 cm² A_e as obtained from the ultrasound data was invariably the larger The linear correlation coefficient between the two sets of determinations is 0 86 cm² with a mean difference of 0 24 A_e as obtained from ultrasound data being the larger The values of A_e at rest obtained from the ultrasound data were related to the internal orifice area (A_i) of the valves Thus A_e was 1 4–1 6 cm² for L–K 18 (A_i=2 5 cm²) 1 7–2 5 cm² for B–S 29 31 (A_i=4 6 cm²) and 2 5–3 4 cm² for L–K 25 (A_i=4 9 cm²) In the four patients studied both at rest and during work the value of A did not remain constant in a given implant Thus on going from the resting to the working state the value of A_e increased 16% and 3% in patients 1 and 9 respectively and decreased 50% and 8% in patients 6 and 8 respectively

Fig 3 presents the results of the in vitro tests For each experimental point the values of the pressure gradient and the flow rate were inserted into eq 3 to obtain A_e The mean values of A were thus found to be 1 9 and 3 0 cm² for L–K 18 and B–S 31 valves respectively A graphic comparison of plots of eq 3 using the mean values of A_e and the obtained experimental points demonstrates a quadratic relationship between pressure gradient and flow rate in the in vitro tests (Fig 3)

The index e_1 (Table I) defined as A_e/A_i represents the fraction of the inner orifice area that is occupied by the blood stream In this study e_1 is a useful indicator of the reasonableness of the values obtained for A_e In the in vitro tests the values of e_1 amounted to 0 76 and 0 65 for L–K 18 and B–S 31 valves respectively In the disc valve implants the values of e_1 obtained from the ultrasound data ranged from 0 28 to 0 72 for L–K and from 0 37 to 0 63 for B–S implants (Table I) Thus for each of the two valve types the values of e_1 obtained in implants were consistently smaller than those obtained in the in vitro tests

Another index e_2 defined as the ratio of A_e and overall valve area (A_o) may be useful as an indicator of implant performance as it relates the magnitude of the obstruction to the available tissue area (assuming that available tissue area is identical to overall valve area) Physically e_2 represents the fraction of available tissue area occupied by the blood stream The mean values of e_2 at rest were 0 38 0 24 0 29 and 0 36 for B–S 29 B–S 31 L–K 18 and L–K 25 implants respectively

DISCUSSION

Determination of the obstructive qualities of mitral disc valve implants with ultrasound requires that Toricelli s law is valid for the flow in implants and that the position of the ultrasound probe is such that cos θ=1 in the Doppler equation Failure to meet either or both of these requirements will result in overestimation of A_e (6) Toricelli s law is generally valid for the flow in short obstructions when the Reynolds number is sufficiently large The in vitro tests were performed at Reynolds numbers in the range that can be expected in mitral disc valve implants and the results of those tests indicate that Toricelli s law is valid

There may be alterations of valve geometry in disc valve implants due to fibrin deposits etc and also inlet and outlet configurations (of left atrium and left ventricle) that deviate considerably from those in the in vitro tests yet it seems likely that Toricelli s law is approximately valid for the flow in

implants Errors in the ultrasound method are therefore likely to be due to a failure to achieve a probe position where $\cos\theta=1$ The geometry of disc valves is such that the maximum velocity vectors can be expected to have a number of different directions (in vitro observations of the flow in L–K valve demonstrated four major diverging jets) this enhances the likelihood of finding a probe position where $\cos\theta=1$ The audio-manual scanning procedure used to locate the optimum probe position is simple but relatively insensitive On line maximum frequency followers have been described (1) incorporation of such instruments in the ultrasound system may be of value in determining the optimum probe position more reliably Nevertheless since there are only a limited number of windows in the thorax that will allow the ultrasonic beam to reach the region of maximum velocity it may not be possible to achieve a probe position in all patients where $\cos\theta=1$

Discrepancies in the values of A_e obtained from the ultrasound data vs the manometric data can also reflect errors in the manometric method In the manometric method the left atrial pressure is in essence measured via a compliant catheter consisting of the flow channels between the tip of the right heart catheter and the left atrium Damping of pressure waves in these flow channels can generally be expected to effect overestimation of the gradient and thus underestimation of A_e (eq 5) Patients with powerful atrial contractions can represent exceptions damping of the left atrial pressure rise due to the atrial contraction can result in underestimation of the gradient during the atrial contraction Patient 8 resting is an example of such a phenomenon In this patient the time course of the maximum frequency shift displayed the effects of the atrial contraction distinctly whereas similar effects were absent in the constructed pressure gradient Thus in patient 8 A_e as obtained from the ultrasound data is likely to be the more correct value

Bjork et al (2) and Book (3) studied a group of 24 patients with B–S mitral disc valve implants (18 B–S 29 31 and 6 B–S 27 note that B–S 29 and B–S 31 differ only in the size of the sewing ring) using in part transseptal catheterization to obtain the left atrial pressure The mean valve area in the group as calculated from the Gorlin formula (4) was reported to be 2 6±0 84 cm² at rest and 3 3±0 99 cm² during exercise The Gorlin formula uses a constant of 31 whereas 51 7 is used for the calculation of A (eq 5) yielding a tentative correction factor of 0 6 The Gorlin formula also uses the approximation $\sqrt{\overline{\Delta P}}$ (square root of mean gradient) rather than the theoretically correct term $\overline{\sqrt{\Delta P}}$ (6) If it is assumed that the appropriate correction factor for the latter situation is 1/0 87 (see Appendix) the final correction factor becomes 0 69 Thus correction of the mean valve area of 2 6 cm² at rest yields $A_e=1$ 8 cm² which compares favorably with the mean A_e of 1 9 cm² at rest as calculated from the ultrasound data in patients 1–5

For a given valve type (L–K or B–S) the in vitro value of e_1 can be expected to be approximately constant for the various sizes because of geometric similarity The L–K and B–S valves differ somewhat in construction and this probably accounts for the difference in the values of e_1 obtained in the in vitro tests of these two valve types The in vitro tests were performed under rather ideal conditions and it seems reasonable to expect lower values of e_1 in implants than the in vitro values This expectation is supported by the findings of others (2 3) who reported the largest valve area of 3 3+0 99=4 29 cm² Correction of this area with the factor 0 69 allows the calculation of $e_1=0$ 64 which is lower than the value of e_1 found in the in vitro tests of B S 31 In the present investigation the highest values of e_1 were also lower than the corresponding in vitro values Since errors in the ultrasound method will result in overestimation of e_1 these considerations indicate that the values of A_e obtained in implants are reasonable as no gross overestimation of A_e is revealed

It is noteworthy that the valve area calculated from the Gorlin formula has no particular physical representation when applied to prosthetic valves thus A_e appears to be a more useful parameter upon which to base performance ratings It should also be noted that the practice of using the approximation $\sqrt{\overline{\Delta P}}$ rather than the theoretically correct term $\overline{\sqrt{\Delta P}}$ can result in significant errors in area determinations when the gradient vanishes before the end of diastole (see Appendix)

In patient 6 the value of A decreased 50% on changing from the resting to the working state This finding indicates that the obstructive characteristics of a disc valve implant are not entirely defined by a single value of A_e The finding may be due to alterations in the configuration of the left atrium and left ventricle that may take place on changing from the resting to the working state

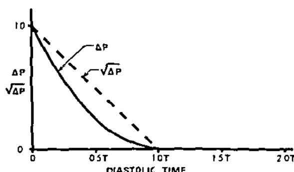


Fig 4 Hypothetical mitral pressure gradient Ordinate is normalized ΔP=pressure gradient T_e=diastolic time where gradient vanishes A_e=effective valve area

Evaluation of the integrals will demonstrate that r is only dependent upon n Some corresponding values of r and n are

n	r
0 5	0 98
1 0	0 87
1 5	0 71
2 0	0 61

These figures demonstrate that when an end-diastolic gradient is present ($n<1$) r approaches unity whereas when the gradient vanishes before the end of diastole ($n>1$) r can become significant

CONCLUSIONS

The results of the present investigation indicate that non invasive ultrasound doppler data can be substituted for invasive manometric data in the determination of the obstructive characteristics of mitral disc valve implants Further studies are necessary to determine whether a technically more sophisticated scanning procedure is necessary Combining the ultrasound method described here with a non invasive method that determines mitral flow rate may well result in a wholly non invasive method for the determination of the obstructive characteristics of mitral disc valve implants

APPENDIX

The ratio of the terms $\overline{\sqrt{\Delta P}}$ and $\sqrt{\overline{\Delta P}}$ depends upon the shape of the curve representing ΔP By choosing a shape that is similar to that often encountered in patients with mitral disc valve implants one can demonstrate the nature of the inaccuracies introduced when the approximation $\sqrt{\overline{\Delta P}}$ is used to calculate valve areas

Consider a mitral pressure gradient ΔP the square root of which decreases linearly with time so that the gradient vanishes at diastolic time T_e (Fig 4) Let time be T and total diastolic duration nT The ratio (r) of the two terms is then

$$r=\frac{\overline{\sqrt{\Delta P}}}{\sqrt{\overline{\Delta P}}}=\frac{\frac{1}{nT_e}\int_0^{nT}\sqrt{\Delta P}dT}{\sqrt{\frac{1}{nT}\int_0^{T}\Delta P dT}} \qquad [6]$$

ACKNOWLEDGEMENT

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Ultrasound as a Complementary Diagnostic Method in Deep Vein Thrombosis of the Leg

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ABSTRACT The ultrasound method for detection of deep vein thrombosis (DVT) of the leg has been used in a study comprising 47 patients This method was compared in the same group with a clinical evaluation and was controlled by venography Clinical evaluation gave a correct diagnosis in 28 (59 6%) of 47 patients, false positive or false negative in 19 (40 4%) With the ultrasound method, the correct diagnosis was made in 41 (87 2%) of 47 patients, false positive in 3 (6 4%) and false negative in 3 (6 4%) No cases were missed of thrombosis situated proximal to the popliteal vein The method was less accurate in cases of thrombosis situated in or distal of the popliteal vein the 3 patients in whom a false negative diagnosis was made, belonged to this group Since the ultrasound method has been shown to give few false positive and false negative results, it should be suitable as a screening procedure in patients with symptoms suggestive of DVT

The diagnosis of deep vein thrombosis (DVT) is difficult to establish Hicks (5) among others has shown that only about 1/3 of patients with acute DVT present clinical symptoms In 2/3 of patients developing pulmonary embolism this is the first clinical evidence of thromboembolic disease (1) All the common laboratory methods for the diagnosis of DVT (venography ^{125}I fibrinogen method venous plethysmography) have disadvantages implying that complementary methods should be valuable

In recent years the ultrasound technique at present widely used for foetal monitoring in obstetrics (7) has been applied to a limited extent when diagnosing peripheral arterial disorders (3) and venous disorders in cases of suspected DVT In the latter connection the most important work has been published by Siegel et al (10) who have also employed the method for evaluation of venous insufficiency in the post thrombotic syndrome

When using the ultrasound technique a signal with a frequency of 2–15 mHz is directed via a cutaneous transducer towards the structure to be examined When the signal is reflected against mowing structures e g blood corpuscles or heart valves the frequency is altered and on reaching the receiver the shift in frequency is registered as a sonic signal The amplitude of the signal is proportional to the shift in frequency and accordingly yields information about e g the velocity of blood flow in an artery The signal can also be registered and recorded graphically

Harmful effects of ultrasound have been searched for As shown by recent studies (12) exposure times of more than 24 hours can be used without proven risk at a radiation level of 10 mW/cm^2 which is usually employed in diagnosis of DVT

MATERIAL AND METHODS

The present investigation comprises 47 patients who either attended the Emergency Department Sodertalje Hospital or developed symptoms suggestive of DVT during hospitalization The criterion for entering the study was the presence of distinct X ray findings either indicating or ruling out DVT

The patients were first examined with regard to 13 symptoms related to DVT (Table I) whereafter a preliminary diagnosis was made from the case history and the clinical findings The patients were then examined by the ultrasonic method The clinical examination and the ultrasonic test were both carried out by the same investigator Venography according to the technique of Greitz (4) was performed in all cases after the ultrasound examination and evaluated by the radiologist on duty The venography served as the basis for evaluating the accuracy of the ultrasound diagnosis of DVT The apparatus

Table I *Incidence of clinical symptoms of DVT*

	Positive venography		Negative venography but DVT suspected	
	n	%	*n*	%
Swelling >½ cm difference in circumference between the calves	19	79	16	70
Spontaneous pain in the calf	18	75	20	87
Increased consistency of the calf	18	75	11	48
Pitting oedema	15	63	15	65
Deep palpatory pain in the calf	14	58	17	74
Venous congestion	8	33	7	30
Temperature >37 5 C	8	33	4	17
Palpatory pain in the planta pedis	8	33	4	17
Leucocytosis >10 000/mm³	7	29	6	26
Hohman s sign positive	7	29	9	39
Cuff test positive (pain in the calf when a cuff over the thigh is inflated to 150 mmHg)	6	25	7	30
Discoloured leg	6	25	10	43
Cough or pathologic chest X ray	5	21	3	13
No of pats	24		23	
No of symptoms	6 3	(S D 2 1)	5 7	(S D 2 2)

used for the ultrasound examination was a Sonicaid pocket ultrasound detector (Teleinvest Company) with a frequency of 2 mHz and an effect of 10 mW/cm² The audible signals only were considered

Technique

With the patient in the supine position the transducer was placed above the femoral artery which was easily identified even in patients in whom the pulse was difficult to palpate By moving the transducer somewhat medially the point was reached where the blood flow in the femoral vein could best be registered The spontaneous flow rising and falling with the respiration was often heard It was easily distinguished from the arterial flow which was usually heard simultaneously Compression of the musculature of the thigh with the hand induced a sudden increase in the velocity of the blood flow in the vein which facilitated the examination Diagnosis of DVT was made if no sound signals or a greatly impaired flow over one leg compared with the other were noticed during local compression of the muscles The patient was subsequently examined in the prone position over the popliteal vein The site with the best arterial flow sound was located The venous flow was most often heard at the same site In the event that a small thrombosis is present in one of the deep calf veins blood flow might still be adequate via the other veins In such cases a normal resting flow sound signal will be heard over the popliteal vein With the transducer over the popliteal vein the muscles of the calf were successively compressed more distally The level at which the most distal compression induced a sonic flow signal over the popliteal vein was noted DVT was considered to be present if a difference of more than 5 cm in this level was recorded between the legs Flow signals can rarely be heard over the posterial tibial vein The absence of sonic flow signals from the posterial tibial vein was not considered to be suggestive of DVT in the present study

RESULTS

A total of 47 patients were investigated Venography showed DVT in 24 patients (51 1%) and was negative in 23 (48 9%) Among the positive DVT patients 15 (31 9%) had high thrombosis (proximal to the popliteal vein) and 9 (19 2%) low thrombosis (in or distal of the popliteal vein)

None of the 13 clinical symptoms considered were found to be significantly more common (χ^2 test $p>0$ 01) in patients with venographically verified DVT than in patients with negative venography The number of symptoms in both groups was essentially the same (Table I) Hohman s sign one classic symptom in DVT tended to be more common in the negative group but the difference did not reach a statistically significant level (χ^2 test $p>0$ 01)

In an attempt to differentiate DVT clinically from other causes of the symptoms a false positive diagnosis was made in 16 patients (34%) false negative in only 3 (6 4%) thus leaving a numer of 28 patients (59 6%) correctly diagnosed

By means of the ultrasound method a correct diagnosis as to presence or absence of DVT was

made in 41 patients (87 2%) A positive diagnosis was made incorrectly in 3 patients (6 4%) and an incorrect negative diagnosis in 3 (6 4%)

The ultrasound diagnosis was correct in all (100%) of the 15 patients with high thrombosis and in 6 (66 7%) of the 9 patients with low thrombosis

With the aid of the ultrasound findings at the two levels investigated attempts were also made to establish whether the thrombosis was situated proximal to distal of or in the popliteal vein The ultrasound method indicated the correct location in 13 (86 7%) of 15 patients with high thrombosis and in 4 (44 5%) of 9 with low thrombosis

DISCUSSION

Despite the lack of specific symptoms more than half of the patients with DVT were diagnosed clinically probably because other factors are important in forming the clinical impression The constellation of symptoms and their intensity for instance may influence the investigator even though they were not recorded in the present investigation The few false negative cases identified clinically in this study may be explained by the selective nature of the patient population All had symptoms when consulting a doctor Patients with silent DVT' in whom the diagnosis would have been more difficult are thus not represented here The ultrasound method made it possible to rule out DVT in 30% of the patients with suggestive symptoms The findings in this respect correspond well with those made earlier by among others Sigel et al (10)

A firm diagnosis could not be made in all DVT negative patients Several developed thrombophlebitis One patient had a Baker s cyst The most probable diagnosis in most of the DVT negative patients was a minor muscular rupture

Considering the patients in whom an incorrect diagnosis was made it is evident that the method is less accurate if there is a small distal thrombosis All false negative cases belonged to that category An explanation for this is that blood flow via remaining patent veins is sufficient to create a sonic flow signal over the popliteal vein which cannot be distinguished from signals recorded on the contralateral side Furthermore blood flow signals may arise after recanalization of the thrombosis The patient should therefore be examined in the acute stage It should also be remembered that other bypassing superficial veins negative diagnosis A sonic flow signal may be obtained despite DVT of the femoral vein if blood passes via the long saphenous vein into a patent saphenofemoral junction In the calf blood may pass e g via the short saphenous vein into the popliteal vein This possibility should be considered in particular in patients with varicose veins or incompetent perforating veins

It should not be forgotten that the investigator may inadvertently induce a pulmonary embolism when compressing the muscles of the thrombosed leg during the ultrasound examination although no such complications were noted during the present study When the technique is used correctly this risk does not seem to be greater than that involved in compression of the muscles during clinical examination or normal walking

The ultrasound method should be regarded as a complement to the clinical examination for screening of patients with symptoms suggestive of DVT It is quickly accomplished cheap without discomfort to the patient and can be repeated The method can be used at the bedside because the apparatus is easily transportable Furthermore because the ultrasound method gives few false negative results it should be suitable as a screening procedure in DVT particularly in cases where venography cannot be performed (e g patient refusal early pregnancy technical difficulties) and should be considered in such circumstances The method should also be of value in studying the course of DVT during therapy Further studies are required both for the evaluation of the ultrasound method in this respect and as a screening method for patients with increased risk for DVT e g postoperatively

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Computer-based ECG Analysis in Acute Myocardial Infarction

A Comparison between Two Computer Programs for the Detection of Ventricular Arrhythmias

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ABSTRACT A routine bipolar ECG has been recorded on magnetic tape in 12 patients with acute myocardial infarction The recording was started 2–8 h after the onset of symptoms and lasted for 12 h Minute by minute classification from a paper chart write-out revealed ventricular ectopic beats (VBs) in all the patients Ventricular tachycardia was seen in 6 patients and 2 had ventricular fibrillation one of whom died The tape recordings were analysed by a non interactive arrhythmia monitoring system The accuracies of two different algorithms for detecting ventricular arrhythmias were compared Program I was based on the comparison of all beats with a running average complex and the correlation of abnormal beats with a typical VB waveform Program II implied the separation of complexes with different morphology on the basis of a five-parameter description The diagnosis of a VB was based on discriminant functions and rhythm criteria A total of 2775 VBs were diagnosed out of which programs I and II correctly diagnosed 48 and 60% respectively With program I only 34% of the alarm situations due to VBs were recognized, whereas 50% were correctly identified by program II False positive VBs amounted to 0 21 and 0 07% respectively, out of a total of 631200 complexes and gave rise to false alarms corresponding to 3/4 of all alarms with program I and about 1/3 with program II—misdiagnosed alarm situations are not included in these figures However one patient with a technically bad recording contributed to more than 50% of the false alarms with both programs Following this study and after minor modifications an arrhythmia monitoring system based on program II has been taken into routine use in our Coronary Care Unit

Continuous ECG monitoring is an important part of the care of patients with acute myocardial infarction (AMI) and in other critical situations Conventional ECG monitoring with nurses watching oscilloscopes is relatively inaccurate (9) and a proper quantification is almost impossible in patients with frequent arrhythmias Automatic alarm systems based on heart rate usually produce a high frequency of false alarms in patients with artifacts in the ECG To overcome these problems *computer-based systems for ECG monitoring have* been developed So far most systems have been evaluated critically only during short test periods rarely exceeding 1 h per patient but rich in ventricular arrhythmias Such short term monitoring discovered 78–95% of all ventricular ectopic beats (VBs) (1 3 4 6 8 11) Recently results from a clinical long term evaluation of a special purpose arrhythmia computer were published by Vetter and Julian (10) However no data from a beat by-beat evaluation or from the performance in particular alarm situations were presented in that study

In the present study a thorough beat by-beat evaluation of the accuracy of a computer based arrhythmia monitoring system has been performed in patients with AMI The study was carried out in order to gain experience during long term monitoring comparing two methods for VB detection The first program (I) was based on a technique originally described by Feezor et al using an analog preprocessor (2) Program II has been developed in its entirety at our institutions It was found to be

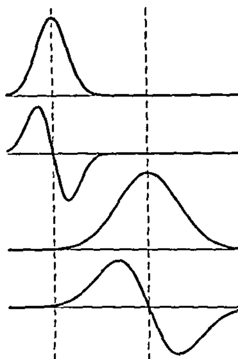


Fig 1 Basis signals used for QRST representation

superior in several respects and is now incorporated into a system which after minor modifications was recently taken into routine use in our Coronary Care Unit (CCU)

TECHNICAL DESCRIPTION

The arrhythmia monitoring system utilizes a minicomput (Datasaab D5/30) with a core memory of 28 K 16-bit ds Eight patients can be monitored simultaneously o on/off and alarm reset controls have been added to xisting bedside units (Elema Schönander) In routine use a beat by beat real time analysis of the ECG is performed Before digitalization the ECG signal is passed through an analog low pass filter (f_{3dB}=50 Hz) to eliminate high frequency artifacts and avoid spectrum aliasing After sampling (100 Hz) the signal is analysed with respect to spikes baseline shifts and powerline noise At a certain level of artifacts the analysis will be blocked for 4 sec Muscle artifacts are suppressed with digital low pass filtering (f_{3dB}=25 Hz) A possible R wave is considered if two first differences with opposite signs and with absolute values above a certain threshold are recorded within 0 25 sec

Program I

This algorithm for VB detection which has been described in more detail elsewhere (6) involves the comparison of all beats with a running average of the complex which dominated at the beginning of the monitoring period (reference complex) If a waveform that has passed the algorithms for detecting R waves and artifacts does not fit properly with the average it is correlated with a typical VB waveform incorporated into the program If the correlation coefficient exceeds 0 8 the complex is marked as ventricular In regular rhythm a 10 % prematurity criterion should also be met before the VB diagnosis is confirmed by the computer

Program II

A waveform recognized as a possible R wave is analysed further and classified as either normal abnormal or artifact This procedure involves the following four steps feature extraction waveform grouping shape classification and final diagnosis

Feature extraction The QRST complex is approximated as a weighted sum of four orthogonal basis functions (Fig 1) The same pairs of functions are used for both the QRS and the ST interval A width parameter has been included for the QRS part of the complex The width parameter of the ST interval as well as the distance between the reference points of the QRS and the ST basis functions are set to fixed standards Consequently five parameters are estimated for each complex the QRS width and the amplitudes of the four basis signals In order to reduce the correlation between the parameters the amplitude coefficients are transformed in pairs into one amplitude and one shape parameter The QRST complex is reconstructed from the above mentioned parameters but only accepted as a true ECG complex if the correlation between the initial and the reconstructed waveform is above a certain threshold

Waveform grouping Complexes with a similar morphology are brought together Each waveform group is characterized by the means and the standard deviations of the five parameters The reference group comprises the waveform that dominates when the monitoring is initiated The grouping of subsequent complexes is based on a distance measure in the five-dimensional parameter space A new waveform group is created when the distance to the existing groups exceeds a fixed limit but this group is considered preliminary until the number of complexes in the group has reached a certain threshold (Fig 2) The parameters of a group are updated whenever a new complex is incorporated A maximum of 10 groups is allowed

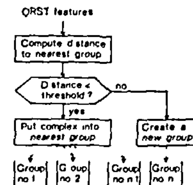


Fig 2 Waveform grouping

for each patient Adjacent groups are merged at regular intervals and a group without new complexes for a certain period is deleted

Shape classification The waveform of the reference group is considered normal Abnormal waveform groups are separated into four categories probable VBs possible VBs abnormal non VBs and essentially normal complexes This classification is based on three linear discriminant functions operating on the parameters of the current waveform (Fig 3) The coefficients of these functions were determined to minimize the probability of misclassification in a large material of different waveforms recorded from CCU patients and manually classified by two physicians

Final diagnosis In this step rhythm data such as prematurity or compensatory pause are combined with the shape classification Consequently this step must await the recognition of the subsequent QRS complex The possible transitions from the waveform type to the final diagnosis are shown in Fig 4 In the present study only the PVBs—below named VBs—were reported by the computer

Power spectrum analysis

In program II a special subroutine is entered when no normal or supraventricular beats have been detected for 5 sec in a noise free signal Asystole is diagnosed if no R waves are detected and the power of the signal is sufficiently low

If these two criteria are not fulfilled the power spectrum of the ECG will be computed Ventricular tachycardia (VT) is considered when the power spectrum shows a narrow peak within 2–4 Hz This diagnosis also follows the detection of more than three VBs in sequence with an average frequency above 2 Hz If the peak in the power spectrum is less marked or above 4 Hz the computer will diagnose ventricular fibrillation (VF) Other conditions will result in an attention alarm if no new complexes are identified within 15 sec

Artifact alarms

In the present investigation it was decided to study only the arrhythmia alarms given for ventricular arrhythmias

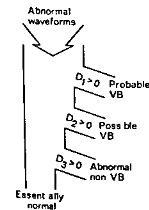


Fig 3 Preliminary shape classification

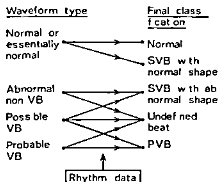


Fig 4 Final classification of abnormal beats SVB = supraventricular beat

These alarm conditions are listed in the method section Program II also presented four artifact alarms which were defined as follows 1) check ECG signal = no ECG complexes detected for 15 sec and VT or VF not diagnosed or more than 50% of the preceding minute blocked due to artifacts 2) VB detection blocked due to artifacts more than 25% of the preceding minute rejected 3) low amplitude = VB detection is prevented if the amplitude of the reference complex goes below 0 6 mV 4) faulty electrode contact = 50 Hz noise in the ECG At any of the first three artifact alarms mentioned above all abnormal waveform groups were deleted Artifact alarms were also given by program I but were not studied in detail

MATERIAL AND METHODS

Patients admitted to the CCU with sudden onset of chest pain less than six hours before admission and with ST T changes in the resting 12 lead ECG were selected The general condition of the patients did not influence their selection A single lead ECG usually with the electrodes placed over the sternum was recorded via the conventional monitoring system A modified Philips 7-channel FM tape recorder was used and recording time was 12 h except in patient 1 who died after 10 h The study did not interfere with routine ECG monitoring No criteria were set on the configuration of the ECG complexes or the signal-to-noise ratio Consequently no exchange or repositioning of the electrodes was made due to the present study In most cases the CCU staff was unaware of the recording of the ECG signal

The attenuation of the ECG signal reproduced from the tape recorder was adjusted at the start of the analysis to obtain a QRS amplitude corresponding to 1–2 mV at the electrodes

The diagnosis of AMI was based on at least two of the following criteria 1) acute onset of chest pain with a duration of more than 15 min 2) typical serial changes in serum CPK values and aminotransferases (ASAT and ALAT) 3) typical ECG changes in the 12-lead ECG Sixteen out of 23 patients recorded proved to have AMI However one recording with peaked P waves and T waves higher than the QRS amplitude was not accepted by

the computer One tape was lost due to a technical mis take One recording was omitted on account of poor technical quality which made a proper interpretation by the physician impossible One patient was excluded be cause of the occurrence of an interfering ventricular rhythm lasting for about 5 h Thus 12 patients remained for the study QRS interval was calculated as the mean of 5 normal complexes from a 50 mm/sec recording made at the start of the recording Bundle branch block (BBB) was diagnosed from a 12 lead ECG taken on admission The P T and QRS amplitudes were estimated hourly from five representative normal ECG complexes The total number of complexes in each patient was estimated from the aver age heart rate computed each minute

Tape recorded ECG data were transferred to paper chart (speed 12 5 mm/sec) and analysed beat-by beat by a physician Abnormal waveforms were divided into three categories VB=ventricular ectopic beat N=abnormal non ventricular beat X=artifact The diagnosis of a VB was based on subjective criteria using prematurity QRST aberration and increase in QRS width A deviation from normal in at least two of these respects was set up as a requirement for the diagnosis of a VB However nar row premature and slightly aberrant beats were usually classified as N In the event of an uncertain interpretation an abnormal complex was classified as a VB When possi ble P wave information was used in the classification of a complex Widened beats with abnormal morphology and unchanged P-Q interval (intermittent BBB) were classified as N This group comprised abnormal beats not fulfilling the VB criteria Premature complexes with otherwise un changed morphology were not considered in this study Abnormal beats disturbed by artifacts were classified as VB or N whereas deranged ordinary complexes were classified as X In patients with episodes of ventricular rhythm or VT only 4 ventricular complexes of each episode were counted Also in a run of *N* beats a max um of 4 were added to the total number of non cular abnormal beats

The physician s interpretation was considered correct and was followed by a beat-by beat comparison with the results from the computer analyses The reasons for false negative and false positive VB detection were analysed with both algorithms for VB detection

A few definitely abnormal complexes overlooked by the physician were detected in the computer analysis When the recording contained several beats of the same configuration the typing of a complex was allowed in retrospect otherwise the complex was considered non ventricular in all cases

The accuracy of the alarms given for ventricular arrhythmias was evaluated by scrutinizing the ECG and the alarm print-out minute by minute False positive alarms due to false positive VB detection were divided into two groups totally irrelevant alarms and alarms with higher priority than the true event False negative VB detection resulted in either the absence of an alarm or an alarm with a lower priority than the real event Ranked after falling priority the alarm situations studied were *a*) VF and VT (VT=more than 3 VBs with an average frequency above 2 Hz) b_1) more than 3 consecutive VBs b_2) 3 VBs in sequence b_3) paired VBs *c*) ventricular bigeminy (3 VBs alternating with normal complexes) or more than 5 VBs/min VF and VT will be reported to gether since program I did not distinguish between these conditions All *a* or *b* alarms whether false or missed were taken into account As to the *c* alarms the following rules were practised 1) An alarm was considered correct if it followed the detection of the first 6 VBs during a running minute 2) If the event was not detected one missed alarm was counted each minute this condition persisted 3) The alarm was not considered if the preced ing minute contained an alarm of higher priority 4) After the correct detection of the alarm it was not studied for the next 5 min An *a* or *b* alarm was considered correct if printed out by the computer within 15 sec after the start of the arrhythmia even though one or more of the complexes were missed in a run of VBs In the beat by beat analysis the first 4 complexes were studied in ventricular rhythm or VT For the correct identification of a *b* alarm the comput er had to report whether 2 3 or more than 3 VBs occurred in sequence

The artifact alarms given by program II were counted and the total time during which the arrhythmia monitoring and the VB detection were blocked was computed and presented at the end of the analysis in each patient Also the total period of irregular rhythm—defined as a relative standard deviation of R R intervals (normal beats) above 10%—was computed and displayed after the monitoring period

The analysis could be restarted manually during the 12 hour period This procedure which implied that a new reference complex was computed and abnormal waveform groups deleted (program II) was usually necessary when there was a sudden change in the configuration of the ECG complex Restarting was also necessary in some patients when minor artifacts had deranged the reference complex resulting in false positive VBs (program I) Any missed or false positive VBs recorded prior to the restarting proce dure were naturally included in the results The ECG was not studied during resuscitation in two patients

Paired *t* test on intraindividual numbers of false positive and negative VBs and alarms was used to evaluate any statistical difference in performance between the two computer programs

RESULTS

Some characteristics of the ECG material are pre sented in Table I Time from onset of symptoms to start of recording varied between 2 and 8 h The total time recorded was 146 2 h or a little more than 12 h per patient The total number of beats recorded was above 600 000 QRS interval varied between 80 and 120 msec Permanent BBB was not seen QRS amplitude showed some variation and was usually above the initial value As to the amplitudes of the ECG the quotients P/QRS and T/QRS varied but never exceeded 0 2 and 0 6 respectively

Sinus rhythm dominated in all patients In patient 1 atrial fibrillation prevailed during the first 2 3 h In

Table I *Some characteristics of the ECG material*

Pat no	Onset of symptoms – start of recording (h)	Recorded time (h)	Total no of beats (×10 ³)	QRS duration (msec)	QRS_{ampl} (initial value =1 0) Min	Max	P_{ml}/QRS_{ampl} Min	Max	T_{mpl}/QRS_{ampl} Min	Max	Irregular rhythm (h)
1	3	10 0	52 5	100	0 7	1 4	0 0	0 1	0 2	0 3	2 6
2	3	12 9	61 9	80	0 8	1 3	0 1	0 1	0 2	0 5	3 9
3	8	11 9	52 5	80	1 0	1 9	0 0	0 1	0 1	0 2	0 1
4	6	12 1	61 1	120	0 6	1 0	0 0	0 1	0 1	0 3	0 5
5	4	12 7	44 5	90	0 7	1 1	0 0	0 1	0 0	0 2	0 5
6	8	12 7	67 4	90	1 0	1 3	0 0	0 1	0 1	0 2	2 9
7	2	12 7	45 0	90	0 8	1 9	0 1	0 1	0 2	0 4	1 2
8	3	12 8	60 9	90	1 0	2 4	0 1	0 2	0 3	0 6	0 5
9	5	12 0	46 8	110	1 0	1 9	0 1	0 2	0 3	0 6	0 1
10	6	12 2	44 1	80	1 0	1 2	0 2	0 2	0 1	0 3	0 1
11	2	12 3	50 0	80	1 0	1 3	0 1	0 1	0 1	0 6	0 5
12	5	11 9	44 5	100	1 0	1 4	0 1	0 1	0 2	0 3	0 1
Total		146 2	631 2								13 0
Mean	4 6	12 2		93	0 9	1 5	0 1	0 1	0 2	0 4	1 1

patients 2 5 6 and 7 irregular rhythm lasted for 0 5–3 9 h and was mainly caused by a sinus arrhythmia In patients 4 8 and 11 premature beats with unchanged morphology resulted in irregular rhythm for about 0 5 h in each Only brief periods of irregular rhythm were seen in patients 3 9 10 and 12

In the beat by beat evaluation (Table II) correct VB identification with programs I and II ranged from 0 to 85% and from 35 to 96% respectively Out of the total number of VBs 2 775 the programs correctly diagnosed 48 and 60% respectively this difference was statistically significant ($p<0$ 05) With program II and patient 8 excluded (due to periods with a high frequency of VBs and artifacts) the percentage of correctly identified VBs in pa

Table II *Comparison between ECG classification by the physician (P) and the computer (C)*
I=program I II=program II

P	VB					Abnormal non VB			Normal		Artifact	
C		VB					VB		VB		VB	
Pat no		I	(%)	II	(%)		I	II	I	II	I	II
1	190	99	52	156	82	38	0	4	2	9	127	15
2	266	74	28	189	71	14	0	1	0	10	2	3
3	15	0	0	8	53	1	0	0	0	0	2	1
4	308	224	73	275	89	574	7	21	11	6	15	19
5	67	4	6	34	51	9	0	1	0	0	1	2
6	54	21	39	19	35	51	0	0	4	23	11	4
7	350	298	85	292	83	11	0	1	4	6	17	7
8	1 321	503	38	559	42	393	9	16	16	20	703	161
9	26	11	42	12	46	0	0	0	341	5	42	11
10	5	3	60	2	40	0	0	0	1	35	17	17
11	61	38	62	25	41	13	0	3	0	13	5	1
12	112	49	44	107	96	0	0	0	14	2	0	0
Total	2 775	1 324		1 678		1 104	16	47	393	129	942	241
Mean	231		44		61							
% of total no of beats	0 44					0 17	0 00	0 01	0 06	0 02	0 15	0 04

Table III *Documented and unreported alarm situations with program I/program II in individual patients*

a = ventricular fibrillation or ventricular tachycardia b = two or more consecutive VBs c = ventricular bigeminy or more than 5 VPs/min

	Correct alarms			False positive alarms: Irrelevant			False positive alarms: Higher priority than true event		False negative alarms: No alarm			False negative alarms: Lower priority than true event	
Pat no	a	b	c	a	b	c	b	c	a	b	c	a	b
1	0/1	2/4	1/2		5/1	3/0		0/1		4/3	1/0	1/0	2/1
2	1/1	2/5	0/1					0/1		9/8	3/2		2*9
3	0/1								1/0	1/1			
4	0/2	19/18	4/5		2/1			1/2	1/0	7/6	1/0	1/0	0/1
5		0/5							1/1	9/2			0 1
6		5/4			2/2					7/7			0/1
7		1/0	4/4		2/1		0/1	0/2		2/3			1/0
8	3/3	32/43	1/1	5/1	161/25		0/1	3/4	2/2	84/68	2/2	1/2	20/24
9		1/2			70/0	4/0				2/1			
10					4/*								
11		2/1			1/1					1/1			1/2
12		1/1	1/4		4/0						3/0		

tients with more than 7-8 VBs/h v as on an average 71-96 (mean 84) With program I the corresponding figures were 28-85 (mean 57)

Abnormal non ventricular beats comprised about one quarter of all aberrant complexes and were rarely interpreted as VBs by either computer program (Table II) Some normal beats v ere falsely classified as VPs by the computer in most patients but the number of misdiagnosed complexes was usually low The percentage of normal beats that were falsely classified as VBs out of the total number of complexes averaged 0 06 and 0 02 by programs I and II respectively For artifacts the corresponding figures v ere 0 15 and 0 04 In a few cases the T wave of a VB was falsely interpreted as a VB and has been included in these figures Pa ient 8 contributed about 70% of all false positive VBs due to artifacts There was however no stati ti cally significant difference between the two programs as to the number of false positive VBs The total number of false VB markings corresponded to 0 21 and 0 07% of the total number of complexes with programs I and II respectively

The result from the recognition of alarm situations in individual patients is given in Table III While unrecognized within 15 sec in program I program II reported VF in a case of rapid VT with a duration of about one minute and simultaneous muscle artifacts VF occurred in patients 1 and 4 This arrhythmia was immediately preceded by a short episode of VT correctly identified in one case by program II In the other patient program II recognized VF after 30 sec This alarm however was ignored in the results With program I "paired VBs and artifacts were reported within 15 sec in the two patients with VF The alarms are summarized in Fig 5 Altogether 79 and 108 true alarms were given by programs I and II respectively On 267 and 52 occasions respectively false positive alarms were produced This difference however was not statistically significant Out of these 4 and

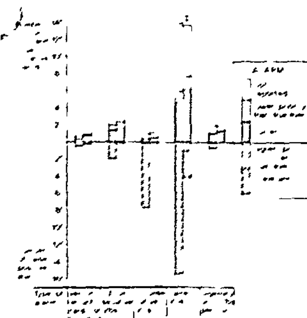


Fig 5 Correct false positive and false negative alarms

Table IV *Inhibition of ECG analysis and artifact alarms with program II*

Pat no	Complete blocking of ECG analysis (min)	VB detection inhibited (min)	Check ECG signal	VB detection blocked due to artifacts	Low amplitude	Faulty electrode contact
1	31	61	29	39	0	0
2	0	1	1	1	0	3
3	37	71	19	37	8	2
4	12	18	9	16	0	17
5	39	52	6	46	2	46
6	13	14	5	12	1	7
7	7	20	2	4	5	0
8	28	83	12	48	5	14
9	10	83	3	8	20	4
10	27	193	17	34	10	27
11	6	143	1	3	9	3
12	0	0	0	0	0	0
Average per pat and h	1	5	0 7	1 7	0 4	0 8

12 alarms respectively were of higher priority than the true event Alarms were lacking in 140 and 107 true alarm situations with programs I and II respectively and in another 29 and 32 cases respectively the alarms were of lower priority than the real event Patient 8 made a major contribution to the number of false positive alarms

With program II ECG analysis was inhibited 0–39 min (mean 18) due to artifacts (Table IV) Corresponding figures for blocked VB detection were 0–193 min (mean 62) Thus VB detection was inhibited about 8% of the total time and this resulted in 161 missed VBs In 131 of these however an artifact alarm was present With program I only 48 VBs were unrecognized due to artifact inhibition of the computer analysis As is seen in Table IV artifact alarms were common in most patients whereas alarms for low amplitude and 50 Hz noise in the ECG occurred less frequently

The reasons for false negative VBs with program I were usually 1) narrow QRS/low correlation with typical VB waveform 2) lack of prematurity and/or compensatory pause and 3) VBs classified as normal beats These reasons explained 90% of all unrecognized VBs

With program II false negative VB detection resulted mainly from the following causes 1) morphology of VB did not fit any of the existing waveform groups 2) failure of parameterization 3) inhibition of VB detection due to artifacts and 4) aberrant premature beats diagnosed as supraventricular Other reasons were noted in 19% of all cases of false negative VB detection

The ECG analysis had to be restarted manually in three patients with both programs and altogether on 20 and 5 occasions with programs I and II respectively

DISCUSSION

The aim of the present study was to investigate the difference in performance between two computer programs and the possible usefulness of computer based ECG monitoring in a CCU Patients with AMI were selected in order to obtain a material with a variety of arrhythmias and because AMI is the main indication for long term arrhythmia monitoring in hospital Only the ventricular arrhythmias were studied in the present investigation since the algorithms for the detection of other arrhythmias were not included in program I As no quality criteria of the ECG signal were specified in advance the recordings from a few patients were very rich in artifacts and it is probable that bad electrode contact or unfavourable positioning of the skin electrodes contributed considerably to the high frequency of artifacts in these patients The ECG noise not only reduced the performance of both programs but probably accentuated the difference between them

With program I artifacts gave rise to a large number of false positive VBs and untrue alarms In a previous study (6) in which program I was tested

on a smaller material (53 000 beats) false positive VBs corresponded to 0 45% of the total number of complexes compared with 0 21% in the present study However more than half the number of false VBs in the previous study originated from abnormal non ventricular beats In the present material such beats caused virtually no false VBs As to the figures for correct VB recognition a decrease from about 80% in the former study to 48% in the present was seen for program I This discrepancy can be partly explained by differences in the ECG materials and in the collection procedures resulting in a larger portion of atypical VBs rejected by program I due to a low correlation with the stored VB waveform The great difference in performance found in comprehensive evaluations of essentially the same program emphasizes the difficulty in comparing different programs for arrhythmia detection on the basis of results obtained from different ECG materials

As to program II the high frequency of artifacts influenced the results in several respects VB detection was blocked at a lower noise level compared with program I Also at an artifact alarm (except faulty electrode contact) all abnormal groups were deleted and since the first complex in a new waveform group was ignored whether of probable ventricular origin or not some VBs were missed for this reason Thirdly ECG noise increased the risk of failure of the parameterization procedure Abnormal groups were deleted if no new complexes were added within a certain period and because the first complex in a group was unreported patients with a low VB frequency showed low figures for correct VB detection In patients with more than one VB/10 min and excluding patient 8 84% of the VBs were diagnosed correctly Considering the low signal quality of the ECG recordings used in this study *this figure must be regarded as satisfactory* The more efficient suppression of artifacts in program II is obtained partly at the cost of a lower detection rate in patients with a low frequency of ventricular arrhythmias However since serious arrhythmias are believed to be less common in such patients this compromise between the demands of sensitivity and specificity seems justified The figure for false VB detection in program II (0 07%) compares favourably with other systems which in short term testing usually have shown values above 0 10%

The alarm criteria chosen in the study are principally the same as those described by Lown et al (7) Multifocal or R on T VBs were not analysed since program I contained no such algorithms

As to the potentially more dangerous arrhythmias about 50% of all incidents of three or more consecutive VBs including VT were immediately detected with a relatively small difference between the programs However, the number of false positive alarms regarding all ventricular arrhythmias studied was markedly higher with program I Before the performance of the present system can be compared with other programs a common base of ECG data has to be established In a few studies conventional ECG monitoring with nurses watching oscilloscopes have been compared with a continuous ECG paper write out In one study by Romhilt et al (9) less than 20% of serious arrhythmias thought to precede VF were detected Holmberg et al (5) found that the nurses detected 6 out of 20 ½ hour periods with VT

The great number of abnormal non ventricular beats seen in some of the patients seems to necessitate programs designed to distinguish between VBs and aberrant complexes of non ventricular origin Table II indicates that both programs under study fulfill this requirement

As can be seen from Table I the amplitudes of the P and T waves may show considerable spontaneous variations in relation to the QRS amplitude but if an acceptable discrimination is obtained at the beginning of the monitoring period there is probably little risk that the ECG monitoring of the present system (program II) will be compromised by P and T waves interfering with the identification of the R wave Alarms for low amplitude were given in several patients and bore no relation to the minimum value of the QRS amplitude reported in Table I Therefore relatively marked *and probably brief amplitude variations in the ECG* complex seem to be rather common This stresses the importance of choosing a precordial lead with a proper QRS amplitude at the start of the monitoring period

The artifact alarms given by program II will become meaningful in clinical routine monitoring when electrode malfunction and other causes of artifacts are immediately corrected Thus experience from routine monitoring with program II shows a lower rate of false alarms with approximately one false message out of seven

Comparing the VB detection of the two pro-

grams the flexible classification scheme of program II has proved superior to the correlation technique used in program I Also the more elaborate procedures for artifact rejection utilized in program II have reduced the number of false VBs to an acceptable level even for ECGs with low signal quality

ACKNOWLEDGEMENT

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The Urinary Sediment in Hydronephrosis

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ABSTRACT Phase contrast microscopy has been used for examination of urinary sediments from 19 patients with hydronephrosis Casts were seldom seen A raised number of erythrocytes was seen only in association with catheter calculi or diabetes A raised number of leukocytes was seen in patients with bacteriuria A striking observation was the finding of a great proportion (64%) of histiocyte like cells in six out of 12 cases in whom a differential count was performed The proportion of histiocyte like cells was small (14%) in cases with bacteriuria or history of urinary tract infection

It has been known since the 19th century that microscopic examination of the urinary sediment can reveal pathological changes in renal disease In 1917 Strauss (6) reported that the proportion of mononuclear cells in the sediment was greater in glomerulonephritis than in pyelonephritis Phase contrast microscopy for differential count of urinary granulocytes mononuclear leukocytes and renal epithelial cells (REP) in different renal diseases confirmed his results (4) The proportion of granulocytes was found to be greater in cystitis and pyelonephritis than in non bacterial diseases such as SLE nephritis and glomerulonephritis Similar findings have been reported by other investigators (1 3 8) Careful examination of the urinary sediment can thus give valuable diagnostic information

The present study was prompted by the chance observation that urinary sediments from some patients with hydronephrosis contained a great proportion of mononuclear cells The aim of this report is to describe and discuss observations on sediments from patients with hydronephrosis since earlier reports on this matter seem to be lacking

PATIENTS AND METHODS

Urine was collected from 19 patients with hydronephrosis and/or ureterohydronephrosis at pyelography The underlying causes were ureteral calculi ureteral stricture hyperplasia of the prostate cancer of the prostate urethral valvule urethral stricture and idiopathic hydronephrosis (Table I) Any history of recurrent urinary tract infection (UTI) prior to the examination was noted Bacterial urinary culture was performed in all cases studied and bacteriuria ($\geq 10^5$ bacteria/ml of urine) was registered as was the serum creatinine concentration In 14 cases voided midstream non morning urine specimens were used In five cases urine was obtained via catheter from the urinary bladder or renal pelvis

Within one hour after sampling 10 ml of urine was centrifuged at 1500–2000 r p m for 10 min and the sediment was immediately examined using a phase contrast microscope At a magnification of ×400 the mean number of WBC and RBC in 10 visual fields was caluculated and presence of casts and their type was noted A differential count of granulocytes mononuclear leukocytes and REP was performed at a magnification of ×1000 using oil immersion The technique used in this work has been described earlier (4) though in the present study mononuclear leukocytes were separated into two groups histiocytes and lymphocytes

Statistical method

Wilcoxon s rank sum test for independent samples was used applying a two-sided hypothesis As level of significance was chosen $p<0\ 05$

RESULTS

Casts were found in six cases (Table I) only one or two hyaline casts in three of them In three cases the number of casts was considerably greater and/or granular casts were present These patients had a raised serum creatinine concentration The number of *RBC* per visual field was raised (15–150) in five cases the urine specimens from three of them were taken via catheter one specimen was from a patient with ureteral calculi and one from a patient with diabetes mellitus The number of *WBC* per visual field was significantly greater in sediments from patients with actual bacteriuria (mean 52 $n=4$) than without (mean 5 $n=15$) How-

Table I *Data on the patients*
UTI=urinary tract infection

Pat no	Age (y)	Underlying disease	Serum creatinine (μmol/l)	Catheter	Previous UTI	Bacteriuria	Casts	RBC/visual field	WBC/visual field
1	66	Idiopathic hydronephrosis	400	–	+	+	–	2	100
2	65	Hyperplasia of the prostate	140	–	+	–	+	2	<1
3	60	Idiopathic hydronephrosis	230	–	+	–	+	<1	30
4	65	Hyperplasia of the prostate	1 000	+	–	+	–	150	25
5	70	Cancer of the prostate	150	+	+	+	–	1	80
6	51	Idiopathic hydronephrosis	80	–	+	–	–	1	5
7	55	Urethral stricture	350	–	–	–	(+)	<1	1
8	61	Cancer of the prostate	1 300	+	–	–	–	<1	15
9	21	Ureteral stricture	100	–	–	–	–	1	8
10	59	Ureteral calculi	120	–	–	–	–	80	15
11	16	Ureteral calculi	90	–	–	–	–	<1	2
12	74	Cancer of the prostate	2 500	+	–	–	–	20	1
13	85	Cancer of the prostate	500	+	–	+	+	15	4
14	25	Idiopathic hydronephrosis	100	–	+	–	–	<1	<1
15	39	Idiopathic hydronephrosis	100	–	+	–	(+)	15	4
16	21	Idiopathic hydronephrosis	90	–	–	–	–	<1	<1
17	18	Idiopathic hydronephrosis	90	–	+	–	–	<1	<1
18	13	Urethral valvule	80	–	+	–	–	<1	<1
19	50	Ureteral calculi	100	–	–	–	(+)	1	<1

ever no significant difference in mean number of WBC per visual field was found between 11 cases with bacteriuria or history of UTI and eight cases without

Differential count could be performed in 12 cases (Table II) was ruled out by paucity of cells in five and by degeneration in two Some cells were degenerated even in the cases in whom differential count was performed such cells were not included the count

The mean proportion of *granulocytes* was 80% in six cases with bacteriuria or previous UTI and 16% in six cases without these characteristics The difference was statistically significant

A great proportion of *mononuclear leukocytes* was noted in seven cases (pats 6–12) In six of them (pats 6–11) the mononuclear leukocytes had a characteristic appearance They were generally slightly larger than a granulocyte and their cytoplasm contained motile granules and often vacuoles (Figs 1 and 2) The granules seemed to be somewhat fewer and larger than granulocyte granules Sometimes the nucleus was elongated and bent or bean shaped (Figs 3 and 4) but more often rounded Almost all of the rounded nuclei were indented at one or more sites (Fig 2) These cells seldom contained more than one nucleus which unlike the nuclear segments of granulocytes (Fig 2) were never connected to each other In many respects these cells fulfilled common cytologic criteria set up for histiocytes Consequently, although their identity was not proven they will be referred to by that designation in the following The mean proportion of histiocytes was 64% in six cases without bacteriuria or previous UTI and 14% in six cases with (Table II) The difference was statistically significant

Table II *Percentage distribution of urinary cell types*
UTI=urinary tract infection

Pat no	Actual or previous UTI	Granulocytes	Mononuclear leukocytes: Histiocytes	Mononuclear leukocytes: Lymphocytes	Renal epithelial cells
1	+	99	0	0	1
2	+	94	0	0	6
3	+	92	2	0	6
4	+	74	5	5	16
5	+	100	0	0	0
6	+	18	76	2	4
7	–	24	76	0	0
8	–	1	97	2	0
9	–	18	82	0	0
10	–	46	32	0	22
11	–	2	94	0	4
12	–	4	0	56	40



g 1 Histiocyte (×2745)



ig 2 Histiocyte (*H*) and granulocyte (*G*) (×2745)



Fig 3 Histiocyte (×2745)



Fig 4 Histiocyte (*H*) RBC and degenerated cells (×1890)

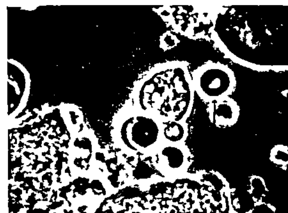


Fig 5 Lymphocyte (*L*) and RBC (×1890)



Fig 6 Lymphocyte (*L*) (×1890)

As *lymphocytes* (Figs 5 and 6) were regarded round cells slightly larger than RBC containing a round nucleus which occupied most of the cell leaving only a narrow rim of cytoplasm at the periphery The cytoplasm often contained a few motile granules Lymphocytes were found in a great number in one case only (pat 12)

Identification of *REP* was based upon criteria described earlier (4) The proportion of REP was judged to be increased (16–40%) in three cases

DISCUSSION

Casts were seldom found even in specimens from patients with a high serum creatinine level This probably reflects the fact that the patients in this study had no known renal disease *RBC* were found in increased number only in cases where factors (catheter ureteral calculi diabetes mellitus) other than the hydronephrosis itself could explain their presence An increased number of *WBC* was correlated to concomitant bacteriuria The proportion of *granulocytes* was significantly greater in cases with previous UTI or concomitant bacteriuria than in cases without This observation paralells earlier reports of an increased number of granulocytes in urine from patients with bacterial urinary tract disease (1 3 4 6)

A striking observation was the abundance of *' stiocyte like cells* in several specimens The origin these cells is obscure They might be associated the hydronephrosis as such since they were ound independently of the cause of hydronephrosis as far as could be judged from this patient population Butterworth (1) states that urine can transform granulocytes into mononuclear cells However in the present study degenerated cells were excluded from the differential count Cells identified as histiocytes did not resemble the degenerated granulocytes found in almost every sediment from patients with UTI Nor does it seem reasonable to assume that a transformation of granulocytes into histiocyte like cells should occur more frequently in patients without than with UTI It seems more likely that an increased excretion of histiocytes takes place in the urine in hydronephrosis and that supervening UTI raises the granulocyte excretion thereby diminishing the proportion of histiocytes The histiocytes seem to correspond well to foam cells free macrophages or cyst phagocytes found in cystic lesions of the breast thyroid prostate and ovary (2 7 9) Papanicolaou and Maddi (5) demonstrated transformation of mammary epithelial cells into free foam cells A hypothesis is that the urinary histiocytes also may be derived from epithelial cells The fact that the histiocytes were observed also in urine from the renal pelvis makes it most probable that they originate either from the kidneys or the renal pelvis It should be added that urinary sediment with cellular patterns like those discussed here have not been observed in about 400 sediments from patients with a broad spectrum of various renal or urinary tract diseases unaccompanied by hydronephrosis

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Membrane Filtration in Microscopical Examination of Urinary Sediment

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ABSTRACT Microscopical examination of the urinary sediment has been performed after membrane filtration and after routine centrifugation, and the results were compared Various quantities of urine were filtered through a membrane with pore size of 3 μm, stained with Shorr stain and made translucent with xylol All granular and cellular casts were counted on a trimmed membrane, 15×20 mm The routine centrifugation was carried out on 10 ml urine at 1500 rpm for 3 min Among 11 patients with glomerulonephritis and recurrent hematuria, casts were found in 9 after filtration but in only 2 after routine centrifugation Casts were detected by the filter method in the urine after angiography of the kidneys in 8 of 12 patients, after centrifugation in only one of them No casts were found in 6 patients with hematuria due to urological disorders and in 21 healthy persons The diagnostic sensitivity of microscopical examination of urine was greatly increased by the filter method This may be due to larger amount of urine examined by the filter method, but an additional cause may be that routine centrifugation destroys red cell casts

In glomerulonephritis with low activity as well as in discrete renal lesions of different etiology repeated routine microscopical examinations of the urine are often needed to reveal pathological constituents of the urinary sediment (1) This problem is accentuated in patients with recurrent macroscopical hematuria in potential kidney donors and in transplanted patients with suspected rejection of the transplanted kidney

The purpose of this study is to describe a rapid and simple method of sediment microscopy which in our hands has considerably increased the diagnostic sensitivity of this procedure

MATERIAL

The study includes 21 healthy individuals (aged 19–38 years) from the medical staff They all had a negative history and negative tests for protein blood and sugar in the urine

Three patient groups were investigated Group I comprised 11 patients with glomerulonephritis and recurrent hematuria Urines from 9 of them were normal one erythrocyte cast each was found in the other two (Table I) They all had normal findings at i v pyelography renal arteriography and cystoscopy The renal biopsy performed after examination of the urine showed an end stage kidney in one patient and mesangial proliferation in the others (Table I) Mesangial deposits of IgA typical of Berger's disease were present in 4 of these patients

Group II consisted of 6 patients with urological disorders and macroscopical hematuria Two had papillomas in the urinary tract four had urinary concrements

In group III consisting of 12 persons urines were investigated before and after abdominal aortography and an additional selective renal arteriography was performed in 8 This group comprised 5 potential kidney donors and 7 patients with various diagnoses (Table II) whose urines had been negative before the angiography

All patients examined had a negative test for albumin in the urine (Albustix)

The ages of the patients ranged from 17 to 62 years

METHODS

Spot urines were examined immediately by the routine procedure for sediment microscopy and by the filter method described below The results were then compared

The routine microscopy was performed on sediments obtained after centrifugation of 10 ml urine at 1500 rpm for 3 min Staining was performed according to the method of Sternheimer and Malbin (5)

In *the filter method* membrane filters of mixed ose acetate and nitrate polymers wi.h a pore siz a diameter of 47 mm (Millip orpo SSWPO 4700) were used Th

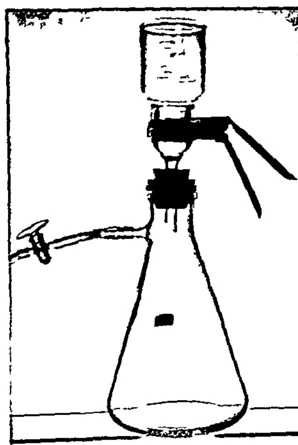


Fig 1 The filter flask with filter holder funnel and forceps

) ine (pH 5 0) and placed on a teflon-faced pyrex filter holder (Millipore Corporation cat no xx1004720) The filter holder was connected to a Buchner filter flask and a water pump was used as vacuum source (Fig 1) Non preserved urine samples were filtered after the membrane and the funnel had been moistened with saline The filtration was continued until it became very slow due to clogging of the filter Care was taken to release the vacuum before terminating the filtration in order to avoid the passage of air through the filter as this may distort cells and casts

The filters were stained with freshly prepared Shorr stain containing 300 mg Biebrich scarlet 75 mg aniline blue 125 mg orange G 25 mg malachite green 1 ml glacial acetic acid 25 mg phosphotungstic acid 250 mg phosphomolybic acid and 100 ml 50% ethyl alcohol All stains were supplied by G T Gurr Products Buckinghamshire England The detailed staining procedure was as follows Freshly prepared and filtered Shorr stain (6 min) 70% ethyl alcohol (15-20 sec) 95% ethyl alcohol (twice 15-20 sec) absolute isopropyl alcohol (2 min) absolute isopropyl alcohol and xylene 1 1 (2 min) and xylene (5 min or until clear) (6)

After the staining procedure the filters were translucent and ready for microscopical examination They were trimmed to a size of 15×20 mm and finally mounted in eukitt under cover glasses All granular and cellular casts on the trimmed filter were counted

RESULTS

Urines from the 21 persons from the medical staff were without granular or cellular casts when examined by both methods The filtrated volume varied from 22 to 100 ml

Routine microscopical examinations showed one red cell cast each in 2 of the patients in group I When the urines were examined by the filtration procedure red cell casts were found in 9

In the 6 patients in group II no cellular or granular casts were observed by either method

During the first 24 hours following roentgenological examination cellular and granular casts were observed by the filter method in 8 of the 12 patients in group III (Table II) while a pathological sediment was observed in only one patient at routine microscopy Six of these patients had a selective renal angiography in addition to the abdominal angiography In all patients the urine sediment was normal 72 hours after the angiography

A pathological sediment observed at routine microscopy was never combined with normal findings by the filter method in the same urine

Table I *Red cell casts in the urine from 11 patients with recurrent hematuria*

Age (y)	Sex	No of casts: Filtration	Centrifugation	Renal biopsy
52	♂	3	0	End-stage kidney
44	♂	4	0	Mesangial proliferation
18	♂	>15	1	Mesangial proliferation
22	♂	>15	0	Not done
26	♀	2	0	Mesangial proliferation
17	♂	0	0	Mesangial proliferation
51	♀	>15	0	Mesangial proliferation
52	♂	>15	1	Mesangial proliferation
29	♀	5	0	Mesangial proliferation
32	♂	0	0	Mesangial proliferation
52	♂	>15	0	Mesangial proliferation

Table II *Cellular and granular casts in the urine from 12 persons after abdominal aortography and renal angiography*

Age (y)	Sex	Diagnosis	Abdominal aortography	Renal angiography	Filtration	Centrifugation
54	♂	Kidney donor	+	+	+	−
51	♂	Kidney donor	+	+	+	+
23	♀	Kidney donor	+	+	+	−
19	♂	Kidney donor	+	+	−	−
40	♀	Kidney donor	+	+	+	−
18	♂	Hypertension	+	−	−	−
33	♀	Hypertension	+	−	+	−
54	♂	Hypertension	+	+	+	−
28	♂	Observation	+	+	+	−
55	♂	Arteriosclerosis obliterans	+	−	−	−
62	♂	Aneurysm of *arteria iliaca*	+	−	+	−
54	♂	Solitary cyst in the left kidney	+	+	−	−
			12	8	8	1

DISCUSSION

The filtration method used here is a modification of the method described by Teitel et al (6) who quantitated cells and casts in urine from patients with various renal diseases using a filter of 1 2 μm After preliminary tests of filters with pore sizes of 1 2 3 5 and 8 μm we found that a pore size of 3 μm gave the best combination of particle retention and maximal filtered volume The intention of this study was not to quantitate casts but rather to see if this method could increase the diagnostic sensitivity of microscopical examination of the urine

In several patients with glomerulonephritis and recurrent hematuria pathological urinary sediments were observed only by the filter method The results in the patients with urological disorders and macroscopical hematuria indicate that the finding of erythrocyte cylinders was not due to artifacts caused by the filtering process The demonstration of red cell casts in patients with recurrent hematuria is of such clinical value that renal biopsy may not be required for diagnostic purposes particulary not in young patients with a typical history of this disease (2)

Renal damage causing a pathological urinary sediment after abdominal aortography has been observed by others (3) In view of the risk of false positive findings the filter method should not be used during the first 3 or 4 days after such an investigation

The difference in diagnostic sensitivity between the two methods did not always seem to be due to the difference in the amount of urine examined A possible explanation may be that centrifugation destroys the casts particularly when the protein concentration in urine is low as the presence of protein seems to stabilize the casts (4)

The indication for using the filter method is doubtful or negative findings at routine examination of the urinary sediment Due to filter clogging the filter method is less advantageous when the sediment is abundant the filtered volume will then be reduced This may explain why no pathological casts were observed in two of our patients with glomerulonephritis Early filter clogging also occurs when the lipid concentration in urine is high and the value of this method is therefore limited in examination of patients with the nephrotic syndrome

It may be concluded that in our material consisting of selected patients the diagnostic sensitivity of microscopical examination of the urine sediment has been greatly improved by the filter method when compared with routine microscopical examination The method is rapid and simple and it may be recommended for routine use

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Regulation of Plasma Aldosterone in Anephric and Non-nephrectomized Patients during Hemodialysis Treatment

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ABSTRACT The relationships between plasma aldosterone and changes in plasma potassium, plasma cortisol, plasma sodium, blood volume and body weight have been studied in 6 anephric and 11 non nephrectomized patients on regular hemodialysis In all patients, the plasma aldosterone concentration decreased during dialysis In the anephric patients, a significant correlation ($p<0\ 001$) was demonstrated between the fall in plasma aldosterone and the fall in plasma potassium (total body potassium depletion) Measurements between consecutive hemodialyses furthermore showed a significant correlation ($p<0\ 001$) in anephric patients between total body potassium repletion (increasing plasma potassium) and the rise in plasma aldosterone In contrast, the potassium and aldosterone changes did not correlate in the non nephrectomized group During dialysis, a decrease was found in all parameters but no correlation was demonstrable in either group between the changes in plasma aldosterone and the fall in plasma cortisol sodium blood volume and body weight The data in the anephric patients emphasize the important role of potassium in the regulation of aldosterone secretion

It is well established that the secretion of aldosterone is stimulated through the renin–angiotensin system (2 16 34 39) the adrenocorticotropic hormone (ACTH) (19 23 32 40) the plasma potassium (3 9 10 17 18 22) and through changes in the sodium and extracellular volume (6 8 15 25 36 41) In normal man the renin–angiotensin system is found to be the main regulator of aldosterone production In anephric man maintained in good condition on regular hemodialysis the absence of the renal renin–angiotensin system makes it possible to study the influence of factors other than the renin–angiotensin system on aldosterone production The hemodialysis treatment leads to regular changes in potassium sodium and body water which are just within the range of physiological variations

However many conflicting data have been reported on the regulation of aldosterone secretion in anephric patients Thus an increase in plasma aldosterone concentration in connection with hemodialysis treatment has been described by McCaa et al (26 27 28 35 49) while a fall was found by other investigators (7 43 51) In some studies (20 27 31) the changes in plasma aldosterone failed to correlate with those in plasma potassium in others such a correlation was found (4 7 14 43 45 49 51 54) A positive correlation to changes in plasma sodium (26 30) could not be verified in other studies (7 20 31 45 55) McCaa et al (30) found that the changes in body volume correlated to those in plasma aldosterone in contrast to several other reports (20 31 45 48 55) Most investigators agree that the plasma aldosterone concentration falls in patients after bilateral nephrectomy and that it is lower in anephric patients than in normal man (20 45 46 47 50 51 52) though a few have found normal or even increased values in anephric patients (27 30 31) A normal increase in plasma aldosterone on ACTH stimulation (29 31 43 49 55) as well as a diminished or no increase have been reported (20 30 45) On the other hand it is commonly agreed that in anephric patients the response of aldosterone secretion to angiotensin II stimulation is absent or reduced (20 29 45 48 49)

These contradictory results concerning aldosterone secretion in anephric patients made it

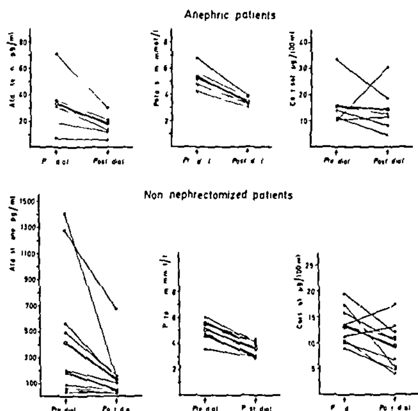


Fig 1 Plasma concentrations of aldosterone potassium and cortisol in 6 anephric and 11 non nephrectomized patients be fore and after hemodialysis

relevant to undertake the present investigation of the relationships between plasma aldosterone con centration and the changes in potassium sodium cortisol blood volume and body weight in anephric 1 non nephrectomized patients on regular hemo ilysis

PATIENTS AND METHODS

The patient population consisted of 17 patients on regular hemodialysis Six patients (4 females and 2 males) with a mean age of 34 5 years (range 16–52) were anephric and had a mean time on regular hemodialysis of 32 5 months (range 9–41) The nephrological diagnoses were chronic glomerulonephritis 2 congenital nephropathy 2 polycyst

Table I *Pre and postdialytic values in 17 patients on regular hemodialysis*

	Predialytic		Postdialytic	
	Mean	S E M	Mean	S E M
Anephric (n=6)				
Plasma aldosterone (pg/ml)	32 50	8 81	18 50	3 45
Plasma potassium (mmol/l)	5 36	0 34	3 50	0 12
Plasma cortisol (µg/100 ml)	16 08	3 58	14 25	3 77
Plasma sodium (mmol/l)	137 00	0 89	135 00	0 44
Blood volume (l)	4 28	0 49	4 03	0 44
Body weight (kg)	50 95	7 23	49 40	7 07
Non nephrectomized (n=11)				
Plasma aldosterone (pg/ml)	414 09	148 79	125 36	52 92
Plasma potassium (mmol/l)	5 10	0 21	3 60	0 16
Plasma cortisol (µg/100 ml)	13 38	1 06	9 33	1 33
Plasma sodium (mmol/l)	136 00	0 93	134 54	0 67
Blood volume (l)	4 98	0 45	4 60	0 40
Body weight (kg)	55 57	5 93	53 75	5 73

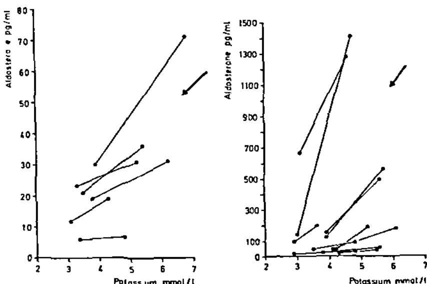


Fig 2 Correlation between changes in plasma aldosterone and plasma potassium in 6 anephric and 11 non nephrectomized patients during hemodialysis Both parameters showed a fall from start to end The arrows indicate the course of the dialysis

ic kidney disease 1 malignant hypertension 1 The 11 non nephrectomized patients (2 females and 9 males) had a mean age of 32.5 years (range 9–55) and a mean time on regular hemodialysis of 17.5 months (range 2–27) The nephrological diagnoses in this group were congenital nephropathy 5 chronic glomerulonephritis 4 malignant hypertension 1 Alport s syndrome 1

All patients were dialysed with disposable parallel plate dialysers (Gambro-Lundia or Boe Dawids) during an average period of 10 hours (range 9–11) twice a week They received a diet containing on an average 0 8 g protein/kg b wt 50 mEq sodium 50 mEq potassium and 800 ml fluid daily They were heparinized during dialysis by single heparin injections the total dose per dialysis ranging from 15 000 to 25 000 IU Before the start of the hemodialysis and following at least 30 min of rest in the supine position blood samples were taken under non fasting conditions from all patients at 8 a m and repeated immediately after the end of the hemodialysis

Plasma aldosterone was measured by a radioimmunoassay (50) plasma cortisol by the competitive protein binding technique (53) potassium and sodium by flame photometry and blood volume by the dilution of ^{131}I albumin (Volemetron®) (24) Body weight and BP were measured before and after hemodialysis Furthermore in 4 patients (2 anephric and 2 non nephrectomized) serial determinations of the plasma concentrations of aldosterone cortisol and potassium were made between three consecutive hemodialyses

The aldosterone antibody was provided by the National Institute of Arthritis and Metabolic Diseases Bethesda USA

The results were evaluated with the Wilcoxon test for paired measurements (38)

RESULTS

The plasma aldosterone concentration in the anephric patients was significantly lower ($p<0\ 01$) than in the non nephrectomized (Table I) During the hemodialysis plasma aldosterone fell in all but significantly only in the non nephrectomized patients ($p<0\ 05$) The fall was more pronounced the higher the predialytic values (Fig 1) In both groups plasma potassium fell significantly ($p<0\ 001$) during the hemodialysis (Table I) A concomitant fall was seen in plasma cortisol most marked in the non nephrectomized group Plasma sodium blood volume and body weight decreased slightly and insignificantly during the dialysis In the anephric patients a significant correlation ($y=0\ 38x+1\ 323$ $r=0\ 982$ $p<0\ 001$) was demonstrated between the changes in plasma aldosterone and plasma potassium during the dialysis (Fig 2) while no such correlation was demonstrable in the non nephrectomized group ($p>0\ 1$) In both groups the changes in plasma aldosterone concentration failed to correlate with the changes in plasma cortisol plasma sodium blood volume and body weight

Measurements made between consecutive hemodialyses showed that the changes in plasma aldosterone and plasma potassium were signifi

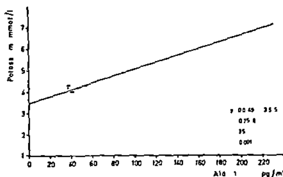


Fig 3 Linear regression between the increase in plasma aldosterone and plasma potassium concentrations between 3 consecutive hemodialyses in 2 anephric patients

cantly correlated during the period of potassium repletion ($y=0.015x+3.515$ $r=0.752$ $n=35$ $p<0.001$) in the anephric but not in the non nephrectomized patients (Fig 3)

DISCUSSION

The plasma aldosterone concentration declined during the course of the hemodialysis in our anephric as well as non nephrectomized patients This finding conflicts with several reports from McCaa et al (26 27 28 35 49) Vetter et al found in one investigation (44) an increase in the aldosterone concentration during dialysis in 4 of 5 anephric patients and in another (43) a decline in 2 patients The [illegible] results do however agree with and confirm [illegible] l that has been reported for anephric patients in other studies (7 43 51) The discrepancies between reports cannot be attributed to differences in the hemodialysis techniques alone as all these procedures in principle correct the electrolyte status and the body volume balance of the patients i e they result in a total body potassium depletion and some reduction of the extracellular fluid volume In anephric patients the influence of the renal renin–angiotensin system can be ignored (5) The well known stimulatory effect of changes in the extracellular fluid volume in normal persons (6 36 41) is dubious in anephric patients since most investigators (20 31 45 48) could not find a correlation to changes in the aldosterone concentration On the other hand a stimulatory effect of acute sodium depletion without variations in plasma potassium or body fluid volume has been demonstrated (26 30) In the present study the blood volume decreased about 6% and the body weight about 3% during hemodialysis in anephric as well as non nephrectomized patients No correlation could be found to the fall in plasma aldosterone concentration The plasma sodium concentration was nearly constant during the dialysis

Probably owing to the normal circadian rhythm (21 33), plasma cortisol decreased in all our patients during the hemodialysis treatment most pronouncedly in the non nephrectomized The stimulatory effect of ACTH on aldosterone secretion is well established (29 31, 43 49 54) In the present investigation the fall in aldosterone concentration during hemodialysis was probably not mainly due to the regulatory function of ACTH as it was much more pronounced than the fall in plasma cortisol Furthermore no correlation was found between the fall in these two plasma concentrations

The fall in plasma potassium (total body potassium depletion) and plasma aldosterone concentrations during the hemodialysis correlated significantly in the anephric but not in the non nephrectomized patients These results conflict with some reports (20 27 31) but agree with many others (4 13, 14 43 45 49 51) They are also in accordance with the view that potassium has a stimulatory effect on aldosterone production in normal man (3 9 17, 18) The present study has not elucidated whether the effect of potassium in the non nephrectomized patients is mediated via the renin–angiotensin system as has been demonstrated by others (1 11 12 22 37 42) At least some modulating factors must be present since the correlation between the fall in plasma aldosterone and plasma potassium only was demonstrated in the anephric but not in the non nephrectomized group The important role of potassium in aldosterone regulation obtains further support from the fact that between the fall in plasma aldosterone and plasma correlation was found in 2 anephric patients between the increase in plasma potassium (total body potassium repletion) and in plasma aldosterone concentration (Fig. 3) In the present investigation, the plasma aldosterone concentration was found to be significantly lower in anephric than non nephrectomized patients This finding is in keeping with several investigations (20 45 46 47 49 50 51) but not with some others (27 30 31) into this problem

In conclusion a close correlation between the fall

in the plasma potassium and plasma aldosterone concentrations was found in anephric patients Furthermore as the increases in plasma potassium and plasma aldosterone correlated significantly this suggests an important role of potassium in the regulation of aldosterone secretion In a previous study (51) we found that this effect may be mediated through alterations in the intracellular potassium concentration

Plasma sodium blood volume and total body weight (as an expression of the changes in total body water) were without significant influence on the plasma aldosterone concentration in any of the patients We therefore believe that the stimulatory effect of these factors on the aldosterone secretion in normal man is mediated via the renin–angiotensin system alone

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Nephrotoxicity in Combined Cephalothin and Gentamicin Therapy

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ABSTRACT Thirty two series of treatment with cephalothin and gentamicin for 5–10 days have been administered to 26 patients An increase in serum creatinine occurred in 6 series Important factors for the renal damage were elevated pretreatment serum creatinine, elevated serum gentamicin and probably a high serum cephalothin In 2 patients the nephrotoxicity was fully reversible, the others died before a dicisive improvement in renal function could be expected In 11 out of 28 treatment series there was a transient drop in serum potassium Since the combination of cephalothin and gentamicin as the primary treatment of life threatening infection has often proved effective, and since short lasting treatment seems to entail only a minute risk of nephrotoxicity in patients with normal pretreatment serum creatinine, we still prefer this treatment in such cases

A few reports on nephrotoxicity—fatal in some cases—in the combination treatment with cephalothin and gentamicin have been published and the renal damage seems to have been acute tubular necrosis (3 4 6 7 11) A publication by Opitz et al (8) has deterred many physicians from using this combination of antibiotics as 8 out of their 14 treated patients developed nephrotoxicity manifesting itself in rising serum creatinine two died However these authors do not make any mention of measuring the serum level of antibiotics and they do not state the frequency with which the serum creatinine was determined Moreover the dose of cephalothin seems to have been high (as a rule a minimum of 12 g/24 hours) Finally the majority of patients were over 70 years old

Moreover hypopotassaemia has been reported during combined treatment with a cephalosporin (cephalexin) and gentamicin (14) However this complication has been reported especially as a side effect of treatment with carbenicillin in combination with gentamicin or in a combination of gentamicin with other antibiotics without the further mechanism being known (2 5 13)

From a microbiological point of view the combination of cephalothin and gentamicin is an ideal treatment of serious infections before the results of bacteriological study are available as also emphasized by Atkinson et al (1) among others In the correct dosage the combination of these antibiotics covers most aerobic and some anaerobic infections Furthermore the administration of these agents is simple and safe as both may be administered intravenously with hardly any risk of anaphylaxis and at intervals of up to 6–8 hours

In Medical Department C Gentofte University Hospital Copenhagen we have been using the combination cephalothin and gentamicin (with close controls of serum gentamicin and renal function) for a number of years with good results and apparently with only a few cases of nephrotoxicity Since however this treatment seems to be falling into discredit in an increasing number of clinics and since there have been no controlled studies apart from that by Opitz et al (8) we decided to carry out a further prospective study of the frequency and seriousness of the nephrotoxicity

MATERIAL AND METHOD

The material comprises 26 consecutive patients (14 males and 12 females) aged 16–81 years who received combined cephalothin and gentamicin therapy for 5–10 days during the period 1 11 1973–1 5 1975 A total of 32 treatment series were administered 4 patients receiving two and one patient receiving three series Most of the patients were suffering from haematological malignancy and the indication for the treatment was life threatening infection of a primarily unknown bacteriology

Gentamicin was injected i v in the course of 5 min The

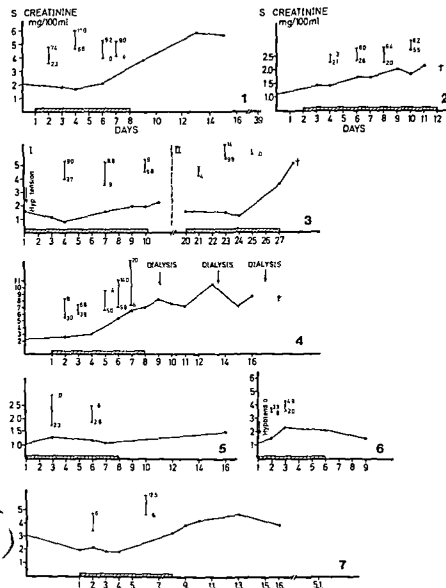


Fig 1 Variation in serum creatinine in conjunction with the treatment with cephalothin and gentamicin (treatment period shaded) in 8 treatment series in which the serum creatinine rose by at least 0 5 mg/100 ml The peak and valley levels of serum gentamicin are also given

initial dose was calculated on the basis of body weight (80 mg for patients over 60 kg 60 mg for patients under 60 kg) while the subsequent doses and the dose intervals during the next 48 hours also depended upon the serum creatinine Later the doses were adapted to serum gentamicin which was determined first on the 2nd–3rd day of treatment immediately before an injection (valley level) and 45–60 min after (peak level) The aim was a peak level of 9–12 μg/ml and/or a valley level of 1–3 μg/ml The determinations were performed by a microbiological method using a bacterial strain sensitive only to gentamicin every 2 or 3 days during the treatment J Bang Antibiotic Department Statens Seruminstitut Copenhagen was helpful in performing the determinations of serum gentamicin Cephalothin was also administered i v as a rule 4 times daily in a daily dose of 4–12 g

Renal function was assessed by serum creatinine determined every other day As a normal level we considered ≤1 3 mg/100 ml in females and ≤1 5 in males An increase in serum creatinine of at least 0 5 mg/100 ml during and up to 2 months after the treatment or until death was recorded as impairment of renal function in conjunction with the treatment In addition serum potassium was determined several times in the course of the study

RESULTS

Twenty patients representing 24 of the 32 treatment series exhibited no impairment of renal function in terms of increase in serum creatinine The mean values of serum creatinine before during and up to 2 months after the treatment also showed no tendency to increase being 1 1 1 1 and 1 0 mg/100 ml respectively

In 8 treatment series (7 patients) the serum creatinine rose by at least 0 5 mg/100 ml in conjunction with the antibiotic treatment Fig 1 sets out the variation in serum creatinine in relation to the treatment in these 7 patients whose histories are reported briefly below

Case 1 A 60-year-old male admitted in 1973 with nephrotic syndrome Renal biopsy minimal changes Myelomatosis was diagnosed a couple of months later At that time the serum creatinine was 5 2 mg/100 ml falling during treatment with prednisone and melphalan to around 2 mg/100 ml When pneumonia supervened combined cephalothin and gentamicin therapy was initiated During this treatment the serum creatinine remained stable at just below 2 mg/100 ml but on the day after discontinuation of treatment it had risen to 4 mg/100 ml and increased further to 5 9 mg/100 ml during the subsequent days falling again to 3.8 mg/100 ml shortly before death one month later Autopsy myeloma kidneys Maximum peak and valley gentamicin levels in serum 12 and 6 6 μg/ml respectively

Case 2 A 63 year-old male with newly diagnosed myeloblastic leukaemia who developed pneumonia at the time of diagnosis Treated with cephalothin and gentamicin On this treatment the serum creatinine rose from 1 4 to 2 1 mg/100 ml shortly before death Maximum peak and valley gentamicin concentrations in serum 8 2 and 5 5 μg/ml respectively

Case 3 A 60-year-old woman who had been suffering from myelofibrosis for many years Treated with cephalothin and gentamicin in Jan 1973

In April 1974 septic shock so that cephalothin and gentamicin were instituted again During this treatment the serum creatinine rose from 1 5 to 2 2 mg/100 ml but a few days after discontinuation of the treatment it had fallen to 1 4 mg/100 ml The maximum peak and valley gentamicin levels in serum 9 4 and 5 8 μg/100 ml respectivly

Ten days later again hyperpyrexia with growth of Gram-negative rods in urine Again treated with cephalothin and gentamicin on which the serum creatinine rose from 1 4 to 5 1 mg/100 ml 7 days later when the patient expired after having gone slowly downhill Maximum peak and valley gentamicin levels in serum 14 and 11 μg/ml respectively Autopsy showed kidneys with normal glomeruli and cadaverous tubules

Case 4 A 63 year-old woman with subacute glomerulonephritis confirmed at biopsy On immunosuppressive treatment the serum creatinine fell from 11 1 to 2.3 mg/100 ml Developed septic fever with marked bilateral pulmonary infiltrations No effect of penicillin ampicillin or methicillin+fucidin Therefore cephalothin and gentamicin were instituted On this treatment the fever subsided but the serum creatinine rose from 2 3 to 8 7 mg/100 ml and the patient had to be treated by dialysis because of hyperpotassaemia Maximum peak and valley gentamicin levels in serum 20 and 6 7 μg/ml respectively The patient deteriorated had persistent attacks of pneumonia to which she succumbed 10 days later Autopsy disclosed Aspergillus pneumonia and microscopic examination of the kidneys showed degeneration of numerous glomeruli with proliferation of the parietal layer The tubules were atrophic and degenerated in several sites Histological diagnosis stage II glomerulonephritis

Case 5 A 53 year-old woman with myelomatosis who developed hyperpyrexia with a large abscess on the buttock so that supplementary treatment with cephalothin and gentamicin was instituted One week after discontinuation of this treatment her serum creatinine had risen from about 1 to 1 5 mg/100 ml The patient deteriorated and died one month later during that period the serum creatinine had slowly increased to 2 3 mg/100 ml The maximum peak and valley gentamicin levels in the serum had been 11 and 2 8 μg/ml respectively

Case 6 A 43 year-old man with chronic myelocytic leukaemia in blastic crisis In the course of 6 weeks he received 3 series of cephalothin and gentamicin because of a suspected septicaemia In the course of the 2nd series the serum creatinine rose briefly from normal to 2 3 mg/100 ml but the patient was hypotensive during the first 24 hours of treatment. Maximum peak and valley gentamicin concentration in serum 4 9 and 2 0 μg/ml respectively No nephrotoxicity in the 1st or 3rd series

Case 7 A 72 year-old man with myelomatosis treated for pheumonia with cephalothin and gentamicin Serum creatinine a few days before the treatment 3 6 mg/100 ml but had dropped to 2 0 mg/100 ml at its institution During treatment and especially during the days after discontinuation the serum creatinine rose to 4 7 mg/100 ml but one month later shortly before death it had fallen to 1 4 mg/100 ml Maximum peak and valley gentamicin concentration in the serum 12 5 and 6 6 μg/ml respectively

Changes in serum potassium could be assessed in only 28 of the treatment series as supplementary treatment with diuretics had been instituted in four cases In 11 of the 28 series the serum potassium fell by more than 0 5 mEq/l to less than 3 5 mEq/l in two patients to less than 3 0 mEq/l A fall in serum potassium was recorded in only one of the eight series with increasing serum creatinine There was no case of an inexplicable increase in serum potassium

DISCUSSION

In cases 5 and 6 we do not believe that the increase in serum creatinine was due to the antibiotic treatment The terminal increase in case 5 must be ascribed to her basic disease (myelomatosis) The short lasting action upon the kidneys in case 6 was presumably of hypotensive nature and indeed soon after this patient was treated anew with cephalothin and gentamicin without any increase in serum creatinine In both these patients the serum gentamicin was at the desired level

Table I *Clinical data on the patients in 26 treatment series without antibiotic induced nephrotoxicity (group I) and 6 series with more or less definitely treatment induced nephrotoxicity (group II)*

	Group I	Group II
No of treatment series	26	6
No of pats	21	5
Mean age (y)	49	63
No of series with pre treatment elevation of serum creatinine	2	5
Mean serum creatinine before treatment (mg/100 ml)	1 1	1 7
Mean duration of treatment with cephalothin (d)	6 8	7 3
Mean duration of treatment with gentamicin (d)	6 7	7 3
Mean total dose of gentamicin (mg)	1 491	1 167
Mean total dose of cephalothin (g)	49 2	72 7
No of series with too high serum gentamicin	4	6
No of series with inexplicable fall in serum potassium	10	1

In 4 patients (cases 1 2 7 and 1st series in case 3) the increase in serum creatinine may possibly have been due to the medication Three of these patients had an elevated serum creatinine at the start of treatment and the increase during the treatment was fairly slight After the treatment serum creatinine fell again to the initial level in two patients showed a decreasing tendency in one whereas case 2 died shortly after discontinuation of the treatment All 4 patients had elevated serum gentamicin levels

In 2 patients (2nd serie in case 3 and in case 4) the increase in serum creatinine seems very probably to have been due to the antibiotic combination Both had impaired renal function at institution of the treatment and died 2 and 10 days respectively after its discontinuation without signs of decreasing serum creatinine These two patients had the highest peak and valley levels of serum gentamicin observed in the whole material

Thus after discontinuation of the treatment serum creatinine fell to levels below the initial in 2 out of the 6 series with more or less definitely treatment induced nephrotoxicity At the worst then it is conceivable that the combination of cephalothin and gentamicin has entailed or been contributory to permanent renal damage assessed by serum creatinine in 4 out of 32 treatment series

Three of the four patients were in the terminal stage of their haematological disease (2 myelomatosis 1 myelofibrosis) and one patient had severe glomerulonephritis All 4 patients died shortly after discontinuation of the treatment and the cause of death was in no case directly related to the neprotoxicity

Table I lists the patients age previous serum creatinine duration of treatment and dose of cephalothin and gentamicin in 26 treatment series without antibiotic induced nephrotoxicity (group I) and 6 treatment series with more or less definitely treatment induced nephrotoxicity (group II)

The patients in group II had a higher mean age ($p<0 05$) and a higher serum creatinine ($p<0 01$) prior to the treatment than the patients in group I (rank sum test)

In all the treatment series of group II the valley level of serum gentamicin was greatly elevated (5 5–11 μg/ml) and in three cases the peak level was also too high (12 5–20 μg/ml) In group I only 4 cases had a high valley level (>3 μg/ml) (3 2–3 6 μg/ml) and only one had an elevated peak level (13 5 μg/ml)

Furthermore the cause of the higher frequency of nephrotoxicity in group II was probably too high a dose of cephalothin as the average dose in the group was 72 7 g compared with 49 2 g in group I In group II high dose cephalothin (12 g/24 h) was given to 3 of 6 patients whereas only 6 of 26 patients in group I received this dose The significance of cephalothin for renal damage has been stressed before (9 10 12)

None of the patients in group II received furosemide during the antibiotic therapy Case 7 received bumetanide 4 mg daily during the last 4 days of the treatment

During the study period another 5 patients were treated with gentamicin combined with ampicillin sodium penicillin or sulphamethoxazole+trimethoprim Both of the patients who received gentamicin and ampicillin developed a transient increase in serum creatinine of more than 0 5 mg/100 ml but both were also receiving bumetanide, one 2 mg daily throughout the antibiotic therapy and the other 4 mg daily during the first 48 hours Incidentally one of these patients had impaired renal function prior to the treatment

Hypopotassaemia was usually mild as already mentioned easily tractable and seemed fully reversible

CONCLUSION

Our experience from treatment of life threatening infection with cephalothin and gentamicin is so good and the risk of treatment induced renal damage in patients with normal serum creatinine so minimal (occuring possibly in one out of 25 series) that in such cases we shall still prefer this treatment However it presupposes a frequent careful control of the serum level of gentamicin serum creatinine and serum potassium and the treatment should probably not be extended over more than 10 days Often it is possible after a few days when a relevant results of bacterial culture is available to continue the medication with just one of the two drugs

In patients with life threatening infection and an existing elevation of the serum creatinine the risk of inducing renal damage seems to be great (occurring possibly in 5 out of 7 series) so that daily control of the cephalothin as well as gentamicin level in the serum and of renal function should be performed moreover the 2 drug therapy should be as short as possible In these cases another broad spectrum antibiotic therapy should perhaps be preferred primarily e g ampicillin+methicillin+gentamicin or carbenicillin+gentamicin although these therapeutic combinations have also been known to induce nephrotoxicity

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The Treatment of End-stage Renal Failure in Insulin-dependent Diabetic Patients

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ABSTRACT Twenty six insulin-dependent patients with diabetes mellitus have been treated for end stage renal failure during a 5 year period The cause of this failure was diabetic nephropathy in 24 cases Eleven patients were temporarily treated with diet alone for their uraemia 23 patients, 7 of whom had previously been treated with diet alone, were treated with dialysis and 9 of these patients were later subjected to kidney transplantation One patient received a kidney graft directly, while his uraemia was treated with diet alone At the time of initiation of the treatment for renal insufficiency, the diabetes had lasted for 22 years and the kidney disease for 5 years (average values) Peritoneal dialysis presented no complications The main problem with haemodialysis was difficulty with the arteriovenous shunt The regulation of the diabetes caused problems in all 10 patients subjected to kidney grafts all required increased doses of insulin Infection and hypertension were prevalent among these patients while rejection of the graft occured in only 2 cases No definite changes were found in the cardiac status retinal changes or BP during treatment The average period of observation for all 26 patients was 10 months The total mortality was 65% approximately half of the deaths were due to cardiovascular causes and about 1/3 to infection The cumulative survival after 3 years was 25% for all patients and 41% after 2 years for the graft recipients The present investigation shows that both dialysis and kidney transplantation are possible in patients with diabetes mellitus and end stage renal failure The goal of future treatment must therefore be kidney transplantation as it makes it possible to offer the patient an acceptable life

The treatment of end stage renal failure in patients with diabetes mellitus presents special problems as a result of the disseminated nature of the disease As a result of experience in recent years (2 3 7 8 10 16 17) from the treatment of kidney insufficiency in this particular group of patients diabetes mellitus is no longer considered a contraindication for active treatment of terminal uraemia in diabetics

The treatment consists of both dialysis and kidney transplantation Nearly all the published reports on the treatment of terminal uraemia with haemodialysis in diabetic patients (2 3 8 16) conclude that the treatment gives the patients an acceptable life only one paper (7) is more sceptical Three reports on kidney transplantation in diabetic patients with end stage renal failure (8 10 17) find the results so satisfactory that the intention is to transplant diabetics on a par with patients not suffering from this disease

The results of kidney transplantation in 5 patients with diabetes mellitus and terminal uraemia were given in an earlier publication (9) from the Transplantation Centre of our hospital The present work follows up this report and also presents the results of treatment of the 26 patients suffering from end stage renal failure and diabetes mellitus requiring insulin who have been treated in the Department of Nephrology during the last 5 years

MATERIAL AND METHODS

The survey has been made retrospectively and two criteria were imposed for inclusion in the study 1) The diabetes mellitus should require treatment with insulin 2) The creatinine clearance should be less than 20 ml/min

The material has been processed in seven sections 1)

Kidney transplantation

Diet Predetermined carbohydrate content

Drugs Immunosuppression using azathioprine and prednisolone Commencing dose of prednisolone was 1 mg/kg b wt /day reduction began one month after transplantation and the maintenance dose of 10 mg/day was reached after 6 months Prophylactic treatment Amphotericine oral tablets and aluminium amino acetate mixture Antirejection treatment Methylprednisolone (30 mg/kg b wt) and antilymphocyte globulin (10 mg/kg b wt) i v

The most important supplements to the above treatment were diuretics antihypertensive agents and digoxin as required

None of the patients were subjected to nephrectomy either before or after transplantation All transplanted patients received cadaveric kidneys and were registered by Scandiatransplant as urgent" i e priority over other recipients having the same tissue type and degree of compatibility

Mortality survival and observation period

The results are shown in Tables I–VII and as curves of the cumulative survival (Figs 2–5)

RESULTS

Twenty six patients 10 women and 16 men were included in the material At the time of the first admission to the Nephroplogical Department Odense University Hospital the average age for the whole material was 47 years (range 26–65) for the women 50 years (range 36–64) and for the men 45 (range 26–65)

Diabetes

On the first admission the average duration of diabetes was 22 years (range 3–42) and of insulin treatment 21 years (range 3–42) The insulin requirement averaged 33 IU (range 12–80) 19 patients received their insulin in the morning and 7 both in the morning and evening The average blood sugar level during the first 5 days of admission was 212 mg/100 ml (range 91–422)

During the first admission 15 patients were on antihypertensive treatment Apart from one patient with a BP of 210/110 mmHg the hypertension was well regulated The average systolic pressure for the group was 160 mmHg (range 210–140) and diastolic 90 mmHg (range 110–70) Eleven patients were normotensive without antihypertensive treatment with an average systolic pressure of 140 mmHg (range 160–125) and diastolic of 80 mmHg (range 90–65) Ophthalmoscopy did not disclose signs of hypertension in 13 patients, 5 had angiopathia hypertensiva and 4 retinopathia hypertensiva none had neuropathia hypertensiva

Two patients had signs of diabetic retinopathy 7 suffered from retinopathia diabetica simplex and 15 from retinopathia diabetica proliferativa

Seven patients had coronary ectasia and 8 radiographic congestion of the lungs detectable by stethoscopy in 6 Eighteen patients had ECG changes indicating compromised heart function Only 3 patients had angina pectoris and none had had myocardial infarction A total of 24 patients (92%) had reduced heart function Only two did not have cardiac symptoms at the time of the first admission Both of them developed signs of heart congestion during haemodialysis treatment

Peripheral arteriosclerosis was present in 15 cases one had no symptoms and information on the others was not available Polyneuropathia was diagnosed in 14 patients could not be demonstrated in one patient and no mention was made of it in the records of 11 patients

Kidney disease

The average duration of kidney disease was 5 years (range 0–13) At the time of the first admission 22 of the 26 patients had a proteinuria of more than 1 g/day the average being 4 5 g/day (range 1 3–10) 5 patients suffered from nephrotic syndrome The creatinine clearance for all 26 patients averaged 6 ml/min (range 0 5–18) At that time the average values of blood urea and serum creatinine were 44 mmol/l (range 11–75) and 995 μmol/l (range 280–2 130) respectively

Twenty-one patients had physical symptoms of uraemia mainly in the form of nausea vomiting tiredness and/or itching Uraemic pericarditis was demonstrated in three cases

Hb concentration at the time of the first admission was 9 0 g/100 ml (range 6 4–12 2)

Twelve patients had significant bacteriuria (>10^6 colonies/ml) during the first admission to the department the predominant strains being Escherichia coli Klebsiella Pseudomonas and Proteus

In 15 of the 17 patients who died the histological results of the post mortem examination were compatible with the diagnosis of diabetic nephropathy In one of them the diagnosis had also been made by kidney biopsy during life Nine patients were alive at the time of commencement of the survey but kidney biopsy had not been carried out in them The kidney disease in 8 of these patients

C	PD	HD	T	OBS
				42
				34
				9
				5
				1
				5
				5
				13
				3
				3
				27
				2
				1/4
				3
				1/30
				4
				7
				6
				16
				12
				15
				25
				15
				2
				8
				8
No of patients 11	11	18	10	

Fig 1 Schematic outline of the treatment groups Horizontal lines broken or unbroken the course of treatment for each patient C=conservative treatment $P-D$=peritoneal dialysis $H-D$=haemodialysis T=kidney allotransplantation OBS=total period of observation (months) from admission to the time of the survey or death

sidered clinically as diabetic nephropathy while the ninth was considered to suffer from chronic interstitial nephritis

Conservative treatment

The average duration of treatment of the 11 conservatively treated patients was 4 months (range 15 days–8 months) (Fig 1) Ten patients could be treated conservatively primarily while one patient had to start with a short period of peritoneal dialysis Six patients had to undergo haemodialysis later one peritoneal dialysis while one who is still alive could be subjected to kidney transplantation directly Three patients remained in this group two of them were alive at the time of the survey one died 14 days after the first admission owing to haemopericardium

The average age for the group was 45 years (range 30–64) 4 patients were women Table I shows the average value of creatinine clearance at the time when the patients were admitted to the group and the average values of insulin requirement and blood sugar for the whole of the observation period

Besides dietary treatment 10 patients were given furosemide 4 antihypertensive agents 3 digoxin and one patient received Resonium®

Hospitalization lasted on an average 27% (range 7–100) of the observation period (Table III)

Peritoneal dialysis

Six women and 5 men with an average age of 44 years (range 26–61) were treated with peritoneal dialysis

The average time elapsed between the demonstration of kidney disease and the start of the peritoneal dialysis was 49 months (range 3–151) The average duration of diabetes at the time when dialysis was commenced was 20 years (range 3–35)

Ten of the 11 peritoneal dialysis patients were subjected to this therapy immediately after admission, while one patient was first treated conservatively for a month Fig 1 shows that 6 patients were transferred to haemodialysis One patient was transferred to conservative treatment after a short period of repeated peritoneal dialysis and was then subjected to transplantation Four patients in this group died

The average level of blood urea just before the first peritoneal dialysis was 50 mmol/l (range 28–70) serum creatinine averaged 1 172 μmol/l (range 415–2300) and creatinine clearance 5 ml/min (range 1–8) The average number of hours/week was 38 (range 9–96) The average levels of blood urea and serum creatinine are given in Table I Technically the dialysis functioned satisfactorily in all the cases There were no instances of peritonitis

The basal dietary treatment had to be supplemented with furosemide antihypertensive agents spironolactone digoxin antibiotics sodium chloride tablets and Resonium® Blood transfusion requirements were modest only 2 patients being subjected to blood transfusion without leucocytes

It was not possible to demonstrate any definite difference in the retinal changes prior to and during peritoneal dialysis (Table II)

The changes in cardiac status were evaluated from the changes in one or more symptoms of the

Table I *Insulin requirement blood sugar blood urea serum creatinine and creatinine clearance different treatment groups (range within parentheses)*

Calculated as average values throughout the total observation period except for blood urea and serum creatinin dialysis groups which are calculated as averages at the start of the dialysis

Treatment	No of pats	Insulin requirement (IU)	Blood sugar (mg/100 ml)	Blood urea (mmol/l)	Serum creatinine (μmol/l)	Creatinin clearanc (ml/min)
Conservative	11	29 (20–48)	198 (115–300)	29 (11–61)	656 (280–1 110)	7 (3–18)
Peritoneal dialysis	11	28 (16–60)	229 (180–330)	37 (27–57)	1 079 (381–1 870)	5 (1–8)
Haemodialysis	18	30 (12–48)	203 (120–311)	26 (21–33)	818 (490–1 310)	

heart disease During peritoneal dialysis the cardiac status was unchanged in 6 patients while there was regression in one and progression in 2 In a further two cases the condition was not stated (Table II)

The insulin requirement fell after the start of the peritoneal dialysis (Table II) The average requirement for the patients in the group immediately prior to peritoneal dialysis was 41 IU daily (range 16–80) and the average requirement during treatment was 28 IU daily (range 16–60) The average blood sugar level before treatment was 214 mg/100 ml (range 112–327) and during dialysis treatment 229 mg/100 ml (range 180–330)

No cases of acute hypoglycaemia or diabetic coma occurred during the peritoneal dialysis

Hospitalization lasted on an average 74% of the observation period (range 41–100) (Table III)

Haemodialysis

Eighteen patients 6 women and 12 men were subjected to haemodialysis Their average age at the start of the treatment was 45 years (range 26–61)

The average duration of diabetes prior to the haemodialysis was 24 years (range 8–42) The average duration from demonstration of the kidney disease up to the start of the haemodialysis was 6 years (range 1 month – 14 years)

Fig 1 shows that 6 of the patients were submitted to haemodialysis directly without previous treatment 6 via conservative treatment 5 via peritoneal dialysis and one via conservative treatment and peritoneal dialysis One patient returned to haemodialysis after removal of the kidney graft

At the time of the survey 9 patients had been subjected to transplantation after being on haemodialysis for an average of 7 months (range 1–16) tients were still on dialysis which had been s 5 and 11 months earlier while 8 patients ha after being on haemodialysis for an averag months (range 1–11)

The average values for blood urea serum c nine and creatinine clearance prior to the first sis were 37 mmol/l (range 22–75) 1233 μ

Table II *Alterations in the diabetic renal l (A) cardiac status (B) and insulin requireme*

The alterations refer either to the condition before ment of the renal disease or to the state in the gro mediately preceding

	Treatment		
	Conservative	Peritoneal dialysis	Haemodialysis
No of pats	11	11	18
A			
Regression			
Unchanged	11	7	14
Progression			
Unknown		4	4
B			
Regression		1	8
Unchanged	11	6	5
Progression		2	5
Unknown		2	
C			
Regression	4	6	8
Unchanged	5	4	5
Progression	2	1	4
Unknown			1

Table III *Time of observation in survivors, non survivors and all patients during their treatment periods, cumulative survival after 1 year and hospital stay as a percentage of the observation time (average values and range)*

Treatment group	Duration of treatment			Cumulative survival after 1 y (%)	Hospital stay (%)
	Survivors	Non survivors	Total		
Conservative ($n=11$)	5 (1–8)	½ (–)	5 (½–8)		27 (7–100)
Peritoneal dialysis ($n=11$)	1 (½–2)	1 (½–3)	1 (½–3)		74 (4–100)
Haemodialysis ($n=18$)	7 (1–16)	3 (1–11)	5 (1–16)	41	33 (15–100)
Transplantation ($n=10$)	12 (4–26)	7 (½–19)	10 (½–26)	62	25 (9–100)

(range 700–1 566) and 3 ml/min (range 1–6) respectively. The average values during the whole observation period for blood urea and serum creatinine at the start of the dialysis are given in Table I.

The patients were dialysed for an average of 15 hours/week (range 1–24). The dialysis was free from complications in 8 patients. The following complications occurred in the other 10 patients: 3 had a tendency to a fall in BP during dialysis, 3 a tendency to excessive fluid consumption, 2 had problems with heart congestion and 8 with access to the circulation. Of these 8 patients 6 were pri[illegible]y dialysed via an internal arteriovenous fistula, 2 via an external Scribner shunt. All of them had clotting problems which required operative procedures. In 2 patients the fistula was corrected operatively with satisfactory results, in 6 it was necessary to establish a new access to the circulation. In two patients it was necessary to have 2 different accesses, in three patients 3 and in one patient 4. In two cases a shunt was established, in the remainder an arteriovenous fistula in the lower arm or thigh.

Patients with shunt problems were treated with haemodialysis for an average of 8 months (range 2–16). The ten patients without problems of access were treated for an average of 5 months (range 1–11); 2 had shunts and 8 fistulae. The 8 patients without dialysis problems were dialysed for 4 months (range 1–11), the others for an average of 8 months (range 2–16).

It was necessary to supplement the basal treatment of the haemodialysis patients with furosemide, antihypertensive agents, digoxin, antibiotics, analgetics, antipruriginosics, psychosedatives and Resonium®.

Seven patients did not require blood transfusion, the remainder received an average of one portion of dextran precipitated blood every 14 days.

The diabetic retinal changes did not change during the treatment compared with the condition on the first admission (Table II). The cardiac status was unchanged in 5 patients, poorer in 5 and improved in 8 (Table II). The insulin requirement during treatment, as compared to that immediately pri

Table IV *Complications to renal transplantation in the 10 diabetic patients*

	No of pats
Complications to renal transplantation and immunosuppressive therapy	
Urinary tract infections	6
Wound infections	6
Pulmonary infections	4
Cardiac insufficiency	2
Rejection of the transplanted kidney	2
Muscular athrophy	2
Urologic complication	1
Other complications after renal transplantation	
Gangrene of the feet	2
Ventral hernia	1
Progression in preexisting disease after renal transplantation	
Diabetes mellitus	10
Hypertension	5
Cardiac insufficiency	1

Table V *Alterations in the average values of creatinine clearance insulin requirement and blood sugar level during the first 12 months after renal transplantation in the 10 diabetics (mean and range)*
Maintenance dosage for the immunosuppressive treatment had been reached after 180 days

	Before transplantation	Days after transplantation				
		1	28	90	180	365
No of pats	10	10	7	7	6	3
Creatinine clearance (ml/min)	-	5 (0 4–8)	52 (29–67)	48 (24–70)	64 (28–73)	63 (60–65)
Insulin requirement (IU)	36 (24–68)	49 (24–80)	75 (64–88)	79 (44–120)	70 (64–72)	61 (32–80)
Blood sugar (mg/100 ml)	203 (120–311)	367 (202–694)	162 (42–313)	262 (120–450)	323 (236–448)	228 (147–296)

or to the first admission became less in 8 was unchanged in 5 and increased in 4 patients (Table II)

The average insulin requirement immediately prior to haemodialysis was 31 IU daily (range 12–48) and during treatment 30 IU (range 12–48) (Table I) The average blood sugar level immediately prior to treatment was 219 mg/100 ml (range 154–422) and during haemodialysis treatment 250 mg/100 ml (range 120–311) (Table I)

The average duration of haemodialysis was 6 months (range 1–16) the hospital stay averaged 33% (range 15–100) of that time (Table III)

No patients were on home dialysis

Kidney transplantation

This group consisted of 10 patients 3 were women The average age at the time of transplantation was 40 years (range 31–50) 41 years for the patients who died in this group and 39 years for the 6 patients who were alive at the time of the survey

One patient was subjected to transplantation directly from conservative treatment while the remaining 9 had all been on haemodialysis for an average of 7 months (range 1–16) (Fig 1 Table III) An average of 3 months (range 1–8) passed from the time at which they were registered with Scandia transplant to the time of transplantation Tissue compatibility was as follows B match 2 B–D match 1 C match 2 C–D match 1 D match 4

The grafts functioned for an average of 10 months (range 8 days – 26 months) Two grafts did not function one owing to thrombosis in the hypogastric and graft arteries The other patient died one week after transplantation One patient rejected the graft twice within the first months and has since rejected continuously (D match) Another patient rejected the graft late the rejection was reversed (C match) The 2 patients in this group who have been treated for the longest time (26 months) were still alive at the time of the survey and had a creatinine clearance of 60 ml/min (B and B–D matches)

The complications to transplantation are shown

Table VI *Causes of death in the different treatment groups*

Treatment group	Causes of death: Cardiovascular	Pneumonia	Sepsis	Malignant neoplasm	Sudden unexpected death	Total
Conservative ($n=11$)	1					1
Peritoneal dialysis ($n=11$)	3		1			4
Haemodialysis ($n=18$)	3	3		1	1	8
Renal transplantation ($n=10$)	2		2			4
Total ($n=26$)	9	3	3	1	1	17

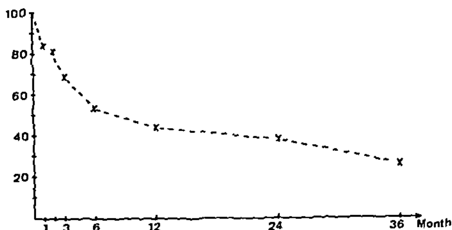


Fig 2 Cumulative survival of all 26 insulin requiring diabetics with end-stage renal failure calculated from the time of the first admission to the Nephrological Department

in Table IV Five patients had an infected urinary tract prior to the transplantation After transplantation significant bacteriuria was demonstrated in 6 patients 4 of whom were infected before operation Infected urine before transplantation in one patient became sterile afterwards whereas sterile urine became infected after transplantation in 2 patients The most important complication was however a severe deterioration of the diabetes occurring in all 10 patients

The blood sugar level during the first 3 months r transplantation was high despite a doubling of insulin dose (Table V) Table V also shows that the insulin requirement with an unchanged blood sugar level after the maintenance dosage for the immunosuppressive treatment had been reached was 1½ times higher than prior to transplantation

Other major complications were worsening of the hypertension already present and a tendency to heart congestion Only one patient had urologic complications corrected after normalization of the kidney function Two patients who had primarily had severe peripheral arteriosclerosis developed gangrene after transplantation (Table IV) One of them was treated by amputating a toe the other by crus amputation

In addition to the immunosuppressive treatment 6 patients were given diuretics 4 antihypertensive agents 3 digoxin and 3 drugs to correct the electrolyte balance

The 10 transplanted patients were observed for an average of 10 months (range 1–26) and 25% (range 9–100) of that time was spent in hospital (Table III)

Mortality survival and observation period

Seventeen of the 26 patients died before the time of the survey Table VI shows the immediate causes of death in the various treatment groups Nine patients (53% of all deaths) died from cardiovascular causes (acute myocardial infarction 4 cardiac insufficiency 3 haemopericardium 1 cerebral apoplexy 1) Three patients (18% of all deaths) died

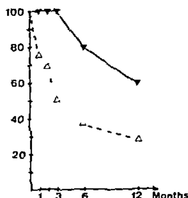


Fig 3 Cumulative survival of the 10 renal transplanted diabetics (▼—▼) and the 16 non transplanted diabetics (Δ Δ) calculated from the time of the first admission to the Nephrological Department

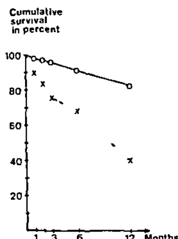


Fig 4 Cumulative survival of the 18 diabetic haemodialysis patients (× ×) calculated from the time of the first haemodialysis O—O=cumulative survival curve of all patients treated in European hospitals with haemodialysis (European Dialysis and Transplantation Association 1970–72)

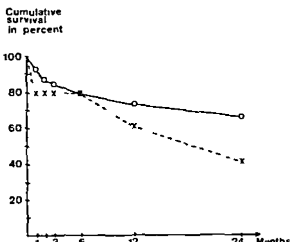


Fig 5 Cumulative survival of the 10 renal transplanted diabetic patients (× ×) calculated from the time of the transplantation O—O=cumulative survival curve of all patients treated in European hospitals with renal allotransplantation (cadaveric kidneys European Dialysis and Transplantation Association 1970–72)

from pneumonia and 3 (18% of all deaths) from sepsis One patient died from gastric haemorrhage as a result of a malignant neoplasm of the stomach which was first diagnosed after initiation of the haemodialysis treatment the patient was then removed from the dialysis programme One patient died suddenly from unknown causes in poor general condition 14 days after the graft had been removed owing to thrombosis of the graft arteries The mortality rate was highest among the haemodialysis patients and next highest among transplanted patients (Table VI)

The cumulative survival for all 26 patients was 44% after 1 year 37% after 2 years and 26% after 3 years (Fig 2) The cumulative survival after 1 year calculated from the first day of admission was 60% for the 10 transplanted patients and 28% for the 16 non transplanted patients (Fig 3)

The cumulative survival for the hemodialysis patients was 41% after 1 year (Fig 4) The cumulative survival calculated from the time of transplantation for the 10 transplanted diabetics after 1 year comprised 62% and after 2 years 41% (Fig 5)

The average period of observation for the total material was 10 months (range 1 day–42 months) for all patients alive at the time of the survey 17 months (range 5–42) for patients who had died prior to this time 7 months (range 1 day–26½ months) Table III shows the average observation time for the 4 treatment groups The observation time is longest for the transplanted group next longest for the haemodialysis group shortest for the peritoneal dialysis group and next shortest for the conservatively treated group

DISCUSSION

End stage renal failure is stated to be a contributory cause of death in half of the patients suffering from juvenile diabetes (17) This fact in connection with improved capacity of treatment has resulted in that several centres now are treating end stage renal failure in diabetics with dialysis (2 4 7 8 16) and transplantation (8 9 10 17)

All our patients were first admitted to the Department of Nephrology at a time when they suffered from severe terminal uraemia At that time their kidney disease had been diagnosed on an average of nearly 5 years earlier and the average age of the patients was 47 years The reason why the kidney disease had not been diagnosed earlier probably has to do with the circumstance that there is a considerable variation in the control of kidney function prior to admission Almost ¹/ of the patients had at the time of the first admission nephrotic syndrome while 85% had a considerable proteinuria This finding is in agreement with the opinion that diabet

ic nephropathy is a primary glomerular disease (1, 5 6 11) which had at this time resulted in a severe reduction in kidney function

The diagnosis of diabetic nephropathy in vivo in our material is based mainly on proteinuria and a severe reduction in kidney function in patients with prolonged diabetes mellitus The routine post mortem histological examination carried out in 15 of the 17 patients who died confirmed the clinical diagnosis

The average time at which the kidney insufficiency occurred in our patients was 21 years after the start of the diabetes This is in agreement with findings of other investigators (2 7 10 16) In addition to the effect on the kidneys 85% of the patients had retinal changes which in 2/3 were of the proliferative type It was not possible to demonstrate any alteration in these changes during treatment This is in agreement with the investigation of Comty and Shapiro (3) whereas others have found improvement after transplantation (10) and a deterioration after haemodialysis (7 16) Other complications to the prolonged diabetes were morbus cordis in 92% and hypertension in 58%

The insulin requirement showed a falling tendency in our conservatively treated patients and in those treated with peritoneal dialysis and haemodialysis when compared with the requirement at the time of the first admission As the blood sugar was unchanged despite this tendency the fall in insulin requirement may be considered to represent an actual fall In contrast to this both Comty and Shapiro (3) and Chazan et al (2) found that the insulin requirement rises after the start of the dialysis while White et al (16) found that this was the case only during the first 4 months on dialysis after which a fall again occurred This difference in insulin requirement after the start of dialysis treatment may be due apart from the above to a possible difference in the dialysis technique among other things the dialysis fluid with haemodialysis was free from glucose in our material and insulin was added to the fluid used for peritoneal dialysis

Our transplanted patients had an increase in their insulin requirement which reached a maximum of twice the pretransplantation level After the maintenance dosage for immunosuppressive treatment had been reached and the kidney function was normalized the insulin requirement still was more than 1½ times the pretransplantation dosage being of the same magnitude as that found by Woods et al (17)

Several investigators (4 12 14 18) have studied the insulin metabolism in the liver and kidneys It appears that the liver plays the main part in the degradation of insulin via insulinase activity while the kidney clearance for insulin varies depending on the total renal clearance It seems also as though a certain amount of sequestration occurs in the tubulus cells possibly as a result of insulinase activity at this site O Brien and Sharpes (12) have studied the clearance of insulin in patients with uraemia before and after kidney transplantation and found in agreement with our study a rise in clearance of insulin after kidney transplantation

The present investigation shows that peritoneal dialysis of diabetics can be carried out without complications in particular peritonitis Chazan et al (2) found that only one of their 33 patients subjected to one or more sessions of peritoneal dialysis developed infection in the cannula wound

Almost 50% of the patients in the haemodialysis group had problems with the route of access to the circulation and required establishment of a new fistula or shunt Only a few patients had a tendency to excessive consumption of fluid and a similar number developed frequent falls in BP during the dialysis Only two patients had cardiac problems during haemodialysis treatment In almost half (44%) the haemodialysis was free from complications White et al (16) found a similar frequency of problems with access to the circulation though their most frequent complication was infection of the fistula All 9 patients of Ghavamian et al (7) had similar problems A previous hypertension produced no therapeutical problems in keeping with the experience of other investigators (7 16) On the other hand, a pre existing morbus cordis deteriorated in 28% of our patients and improved in 44%

Of the 10 transplanted patients only 2 had rejection It has previously been found in our Transplantation Centre that rejection is rare in these patients (9) Later experience has not confirmed this observation Rejection clearly occurs in diabetics All that can be stated is that rejection is rarer in diabetics than in other patients No material is yet large enough to give a definite answer to this question Kjellstrand et al (10) found a frequency of 30% in a material of diabetics with both cadaveric and living family donor kidneys and controls Woods et al (17) in their material of living family donor kidneys found one rejection among 8 transplanted patients

In our material 6 of 10 patients had urinary tract infection after transplantation 4 of these had had urinary tract infection prior to operation None of our patients had been subjected to bilateral neph rectomy before or after transplantation Only one patient had urological problems which were suc cessfully treated with surgery whereas Kjellstrand et al (10) observed urological problems in 7 out of 22 patients transplanted with cadaveric kidneys An improvement was observed in the cardiac status after transplantation in 7 of our 10 patients

The cumulative survival for patients in the pres ent material was 40% after 2 years This is some what lower than that found by Kjellstrand et al (10) who found a cumulative 2 year survival of approximately 65% whereas the 1 year survival was almost equal in the two materials The fact that the cumulative survival in the haemodialysis group was poorer compared with the transplanted patients presumably has its explanation in the fact that 9 (50%) of the patients left the group at the time of transplantation

The mortality among the transplanted diabetics was 40% which is higher than 29% reported by Kjellstrand et al (10) In the haemodialysis group the mortality was somewhat lower than that found by other investigators (3 16) when the period of observation is taken into consideration

The causes of death among the patients in our various treatment groups are distributed in almost the same manner as reported by others (2 7 10 16)—approximately half of the patients died from cardiovascular causes approximately one sixth from pulmonary infection approximately one sixth from sepsis and one sixth from various causes

An interesting observation in the present investi gation is that the transplanted patients did not spend more time in hospital than those treated conserva tively whereas haemodialysis patients and particu larly those having peritoneal dialysis spent some what more time in hospital

It is not known how many patients can be treated in this manner However the number in Scandina via appears to lie somewhere between the number of patients with cystic kidneys and with glomerular nephritis who are actively subjected to uraemic treatment each year Although this number is large we do not consider it impossible to treat severe juvenile diabetes and end stage renal failure in this way in all centres

We had hoped at the start of this survey that it would be possible to state with reasonable certainty which category of patients were suitable for this treatment in particular those who could be expect ed to live reasonably well for a number of years and which category were unable to stand this type of treatment For example whether or not such parameters as age myocardial condition or blind ness could be prognostic and predictive However this relatively small study as well as other similar Scandinavian investigations have shown that no de finite conclusion can be made as to these selective criteria At present it is necessary to continue the treatment of these relatively young patients until we have enough experience to state with reasonable certainty whether or not there are groups which are unable to withstand the treatment

The treatment itself gives the fortunate patient a few good years with a transplanted kidney but everything indicates that the patient will compara tively quickly develop late diabetic complications even though not in the transplanted kidney It is therefore of considerable importance that we have now started pancreatic transplantation in Scandi navia in an attempt to improve the prognosis of these patients In this way the whole of the renal treatment will acquire far better prospects One can only hope that pancreatic transplantation will prove more successful than it has to date and that at least it will prevent the progression of the diabetic vascu lar disease If it is successful there is little doubt that this group of patients will be treated actively in future

CONCLUSION

The present study has shown that dialysis is a fea sible form of treatment of end stage renal failure in patients with diabetes mellitus requiring insulin It is clear that dialysis treatment is only a temporary measure and that kidney transplantation is neces sary as soon as possible in these patients inas much as it offers the patient an acceptable life most of which can be spent outside hospital

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Haemodynamics and Renal Function Following Injection of Bumetanide

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ABSTRACT Haemodynamics and renal function have been investigated in 12 patients with valvular heart disease before and after injection of 2 mg bumetanide in the right heart catheter There were no significant changes in oxygen consumption, arteriovenous oxygen difference, cardiac index heart beats per minute, stroke volume or right and left ventricular stroke work 35 min after the injection whereas pulmonary and systemic arteriolar resistance showed a slight but insignificant reduction Mean pulmonary capillary venous pressure, left ventricular end-diastolic pressure, mean pulmonary arterial pressure and mean pressure in the right atrium were highly significantly reduced after injection, systolic left ventricular pressure showing a significant but slight decrease The creatinine and urea clearances increased considerably during the first 50 min after injection of bumetanide but diminished during the second period to levels somewhat lower than the initial values There was also a marked increase in the clearances of sodium potassium calcium, magnesium and phosphate It is concluded that bumetanide is a very potent diuretic which changes haemodynamic parameters towards normal values

Bumetanide (3 n butylamino-4 phenoxy 5 sulfamoylbenzoic acid) is a relatively new diuretic which differs chemically from others in current use (10 11) The structural relationship to sulfamovl diuretics including furosemide has been discussed (10) A major site of its action is on the ascending limb of Henle s loop but investigations suggest that the drug has an additional action on the proximal tubule (4) The diuretic effects are as powerful as those of the other loop diuretics currently available i e furosemide and ethacrynic acid with an onset and peak of action similar to these (20) Bumetanide in a dose of 1 mg has a diuretic effect equivalent to 40–60 mg of furosemide (2 4 5 7 12) A similar magnitude of total natriuretic response is found after oral and i v administration of the drug (7)

MATERIAL

The material comprised 12 patients 9 women and 3 men with an average age of 55 years All had mitral stenosis complicated by mitral insufficiency in 7 The average roentgenological volume of the heart was 815 ml/m² All the patients were treated with digitalis 11 with digitoxin and one with digoxin Eleven patients were treated with diuretics 7 with furosemide 3 with trichlormethiazide 1 with furosemide and spironolactone All diuretics were discontinued at least 48 hours before the investigation (Table I)

METHODS

Right heart catheterization was performed with a Cournand catheter A polyethylene catheter was placed by the Seldinger technique in the aorta and left ventricle via the femoral artery The examinations were performed in connection with preoperative heart catheterization The patients were informed of the purpose of the study and had given their consent Pressures were recorded in the right atrium the pulmonary artery wedge position left ventricle and aorta Cardiac output was measured according to Fick s principle using a Douglas bag Oxygen consumption was calculated from gas analysis carried out by the micromethod of Scholander Systemic arteriolar resistance pulmonary arteriolar resistance right ventricular stroke work (RVSW) and left ventricular stroke work (LVSW) were calculated

Over a period of 3 min 2 mg bumetanide was injected in the Cournand catheter placed in the pulmonary artery Pressures in the pulmonary artery wedge position and left ventricle were measured at 5 or 10-minute intervals The cardiac output was measured before and 35 min after the injection The pressures in the aorta and the right atrium were measured before and a hour r injecting bumetanide

Table I *Clinical data on the patients*

MS=stenosis of the mitral valve IM=insufficiency of the mitral valve TI=insufficiency of the tricuspid valve AI=insufficiency of the aortic valve slight degree indicated within parentheses

Pat no	Sex	Age (y)	Diagnosis	Heart volume (ml/m²)	Digitalis	Previous diuretic
1	♀	60	MS+IM+(AI)	870	Digitoxin	Furosemide
2	♀	47	MS	590	Digoxin	Trichlormethiazide
3	♂	64	MS+IM+(TI)	1 340	Digitoxin	Furosemide
4	♀	55	MS+IM	1 400	Digitoxin	Furosemide
5	♀	61	MS	610	Digitoxin	Furosemide
6	♂	57	MS	500	Digitoxin	
7	♀	56	MS+(IM)+(TI)	930	Digitoxin	Trichlormethiazide
8	♀	31	MS	550	Digitoxin	Furosemide
9	♀	61	MS+(IM)+(AI)+(TI)	810	Digitoxin	Furosemide spironolactone
10	♀	49	MS+IM	650	Digitoxin	Furosemide
11	♀	60	MS	860	Digitoxin	Furosemide
12	♂	57	MS+IM	665	Digitoxin	Trichlormethiazide
Mean		55		815		

Creatinine and urea clearances as well as the clearances of sodium potassium calcium magnesium chloride and phosphate were measured before and in two clearance periods after the administration of bumetanide Due to the differences in the length of individual clearance periods the times 23 3 and 69 0 respectively are defined as means of the times from injection to the middle of the clearance period (Table IV)

The urinary bladder was catheterized and flushed with 30 ml distilled water An equal amount of air was also carefully instilled in order to empty the bladder as far as possible Sometimes however it was not possible to recover all the water used for flushing when emptying the bladder

RESULTS

The various measurement of pulmonary arterial pressure pulmonary capillary venous pressure systolic left ventricular pressure left ventricular end diastolic pressure and heart rate were analyzed statistically using a linear regression with time as the independent variable The calculated change per hour is given in Table II together with its standard deviation and the significance (Student s *t* test)

The values reveal highly significant reductions of pulmonary capillary venous pressure and left ventricular end diastolic pressure after the injection of bumetanide The mean pulmonary arterial pressure was likewise extremely significantly diminished while the fall in systolic left ventricular pressure was slight and scarcely significant The mean pressure in the right atrium decreased significantly

Table III shows no significant change of oxygen consumption and arteriovenous oxygen difference 35 min after injection of bumetanide Consequently there was no significant alteration of cardiac output or cardiac index Heart beats per minute were very constant and therefore stroke volume was also unchanged A tendency although not significant was noted towards a decrease in pulmonary and systemic arteriolar resistance Mean RVSW and LVSW showed no change

One hour after injection of bumetanide there was a highly significant reduction of mean pulmonary capillary venous pressure and left ventricular end diastolic pressure The mean pulmonary arterial pressure also showed a highly significant decrease while the fall in systolic left ventricular pressure was slight and hardly significant

The creatinine and urea clearances *increased* considerably in the first clearance period after injec

Table II *Calculated changes of haemodynamic parameters (mean±S E M)*

PAP=pulmonary arterial pressure PCVP=pulmonary capillary venous pressure SLVP=systolic left ventricular pressure LVEDP=left ventricular end diastolic pressure HR=heart rate

	Change/h	*p*
PAP (mmHg)	−4 8±0 6	<0 001
PCVP (mmHg)	−2 8±0 5	<0 001
SLVP (mmHg)	−5 9±2 3	<0 05
LVEDP (mmHg)	−3 7±0 6	<0 001
HR (beats/min)	2 8±2 0	NS

tion of bumetanide and diminished in the second period to values somewhat below the pretreatment levels

As shown in Table IV there was a significant increase in the clearances of sodium potassium calcium magnesium chloride and phosphate in both periods after treatment but especially in the first

There was a marked and significant diuresis in the two clearance periods following the injection of the drug

DISCUSSION

As detailed above this investigation revealed important haemodynamic effects of bumetanide—under the influence of the drug all such parameters monitored showed a reversion towards normal values Furthermore the 12 patients had been treated with other diuretics before this investigation These drugs were discontinued at least 48 hours before the haemodynamic examinations but the observed changes might have been even more striking if the investigation of bumetanide had been carried out on patients not previously exposed to diuretics

In former publications the i v bumetanide dose was 3 mg (3 20) and 1 mg (12) A 2 mg dose was chosen in this investigation and bumetanide was injected through the right heart catheter in the pulmonary artery Physiologically this may be regarded as an i v injection and this route is also used in examinations of furosemide (14)

Haemodynamic effects have been investigated after treatment with different diuretics Rader et al (16) performed such examinations before and after injection of mercurial diuretics and found a considerable reduction of pressure in the right atrium and the pulmonary artery The same findings were observed in systemic arterial hypertension after chlorothiazide (18) Stampfer et al (21) found a significant decrease in mean pulmonary arterial wedge pressure mean pulmonary arterial pressure and mean right atrial pressure after treatment with chlorothiazide and hydrochlorothiazide Cardiac index decreased after thiazides but remained essentially unchanged after mercurial diuretics just as in the present investigation of bumetanide

Haemodynamic examinations after treatment with furosemide have revealed a significant reduction of systolic pulmonary arterial pressure (1) and other investigators have found decreases in mean left atrial pressure mean pulmonary capillary venous pressure or left ventricular filling pressure (8 13 14) and mean pulmonary arterial pressure (6 14) A significant decrease in right atrial pressure has been found following treatment with furosemide (8) as in this investigation of bumetanide Several studies on furosemide (1 6 8 13 14) revealed little or no reduction of systemic arterial pressure and cardiac index but in some investigations the changes were slightly significant Our examination of the effect of bumetanide showed a slight but significant decrease in the systolic left ventricular pressure while the cardiac index did not change significantly Summing up the results after treatment with furosemide resemble the haemodynamic effects of bumetanide observed in the present study

After i v injection of ethacrynic acid a considerable fall of central venous pressure and increases in cardiac index and stroke index have been noted in patients with congestive heart failure (17) Another investigation of the effects of ethacrynic acid (19) revealed a significant reduction of mean pulmonary artery systolic and diastolic pressures Cardiac output and stroke volume either remained essentially unchanged or fell after administration of ethacrynic acid in 9 of the 13 patients studied

In the present study a considerable increase was noted in creatinine and urea clearances A significant increase in glomerular filtration rate was also observed in several other studies (3 12 20) whereas the change occurring after bumetanide both during water diuresis and hydropenia was negligible in one study (4)

Our investigation revealed a significant post treatment increase in the clearances of sodium potassium calcium magnesium chloride and phosphate As may be seen from the literature such clearance studies after bumetanide injection have not been performed previously Studies in healthy volunteers have shown that bumetanide increases the excretion of sodium chloride potassium and water with a pattern similar to that produced by furosemide (5) Corresponding results have been found by others (7 12 15) The excretion of calcium and magnesium increased significantly in the study by Sigurd et al (20) in accordance with findings by others (7) A retention of calcium was found 6–12 hours after treatment with bumetanide Bourke et al (4) observed an increase in phosphate excretion during the peak of diuresis

Table I *Clinical data on the patients*

MS=stenosis of the mitral valve IM=insufficiency of the mitral valve TI=insufficiency of the tricuspid valve AI=insufficiency of the aortic valve slight degree indicated within parentheses

Pat no	Sex	Age (y)	Diagnosis	Heart volume (ml/m²)	Digitalis	Previous diuretic
1	♀	60	MS+IM+(AI)	870	Digitoxin	Furosemide
2	♀	47	MS	590	Digoxin	Trichlormethiazide
3	♂	64	MS+IM+(TI)	1 340	Digitoxin	Furosemide
4	♀	55	MS+IM	1 400	Digitoxin	Furosemide
5	♀	61	MS	610	Digitoxin	Furosemide
6	♂	57	MS	500	Digitoxin	
7	♀	56	MS+(IM)+(TI)	930	Digitoxin	Trichlormethiazide
8	♀	31	MS	550	Digitoxin	Furosemide
9	♀	61	MS+(IM)+(AI)+(TI)	810	Digitoxin	Furosemide spironolactone
10	♀	49	MS+IM	650	Digitoxin	Furosemide
11	♀	60	MS	860	Digitoxin	Furosemide
12	♂	57	MS+IM	665	Digitoxin	Trichlormethiazide
Mean		55		815		

Creatinine and urea clearances as well as the clearances of sodium potassium calcium magnesium chloride and phosphate were measured before and in two clearance periods after the administration of bumetanide Due to the differences in the length of individual clearance periods the times 23 3 and 69 0 respectively are defined as means of the times from injection to the middle of the clearance period (Table IV)

The urinary bladder was catheterized and flushed with 30 ml distilled water An equal amount of air was also carefully instilled in order to empty the bladder as far as possible Sometimes however it was not possible to recover all the water used for flushing when emptying the ladder

RESULTS

The various measurement of pulmonary arterial pressure pulmonary capillary venous pressure systolic left ventricular pressure left ventricular end-diastolic pressure and heart rate were analyzed statistically using a linear regression with time as the independent variable The calculated change per hour is given in Table II together with its standard deviation and the significance (Student s *t* test)

The values reveal highly significant reductions of pulmonary capillary venous pressure and left ventricular end diastolic pressure after the injection of bumetanide The mean pulmonary arterial pressure was likewise extremely significantly diminished while the fall in systolic left ventricular pressure was slight and scarcely significant The mean pressure in the right atrium decreased significantly

Table III shows no significant change of oxygen consumption and arteriovenous oxygen difference 35 min after injection of bumetanide Consequently there was no significant alteration of cardiac output or cardiac index Heart beats per minute were very constant and therefore stroke volume was also unchanged A tendency although not significant was noted towards a decrease in pulmonary and systemic arteriolar resistance Mean RVSW and LVSW showed no change

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LVEDP (mmHg)	−3 7±0 6	<0 001
HR (beats/min)	2 8±2 0	NS

Mean right atrial pressure (mmHg)		Mean aortic pressure (mmHg)		Pulmonary arteriolar resistance (dyn/sec cm⁻⁵)		Systemic arteriolar resistance (dyn/sec cm⁻⁵)		Right ventricular stroke work (g m)		Left ventricular stroke work (g m)	
A	C	A	C	A	B	A	C	A	B	A	B
9	−1	116	−16	178	−70	2 378	−389	19 4	−0 8	72 5	−5 0
6	−1	96	4	156	−17	1 756	−104	15 0	3 1	49 7	7 2
3	−2	104	−20	346	10	2 184	−340	24 5	−6 8	47 9	−5 6
10	−7	88	−4	175	−77	1 950	−370	14 0	5 6	55 8	18 6
5	−3	88	−8	139	−96	1 443	243	13 9	−5 4	73 1	−19 4
-1	−2	100	−24	200	−73	2 244	−808	11 7	2 0	56 7	1 2
11	−8	128	−16	55	11	2 127	−697	4 8	13 1	113 9	20 3
5	−5	84	8	168	−38	1 663	−161	9 1	5 8	56 4	25 0
7	−1	88	4	56	35	1 507	459	19 9	−9 5	75 4	−17 8
7	−3	84	4	78	42	1 502	178	12 7	1 5	53 9	6 7
5	−2	108	−36	102	54	1 753	−407	19 8	−5 6	140 0	−58 4
2	−2	76	8	123	−67	1 518	45	9 9	−1 2	65 5	6 7
	−3 1		−8 0		−23 8		−196		0 2		−1 7
	0 7		4 2		15 3		110		1 8		6 5
	<0 001		NS		NS		NS		NS		NS

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Pronounced Deficiency in T-cells and Lymphocyte Chromosomal Aberrations in a Patient with Sarcoidosis, Myelofibrosis and Acute Leukaemia Following Thorotrast Angiography

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ABSTRACT A patient exposed to thorotrast angiography developed sarcoidosis 21 years after the injection and myelofibrosis 13 years later On the latter occasion an extreme deficiency in circulating lymphocytes forming rosettes with sheep erythrocytes (T-cells) was observed and a large fraction of the cells had chromosomal aberrations Acute leukaemia developed 1 year later The multiple clinical symptoms may be related to radiation induced destruction of bone marrow tissue, mutations in haemopoietic cells and depression of cell mediated immunity

Several solid tumours usually of angioendothelial types aplastic anaemia chronic and acute myeloid leukaemia have been attributed to thorium radiation (8) Considering the long interval between the administration of thorium and the manifestation of alleged sequels the causal relationships may sometimes appear doubtful With this reservation we feel justified in reporting the finding of sarcoidosis and myelosclerosis with myeloid metaplasia transforming to acute myeloblastic leukaemia in a man who had been injected with thorium in 1939

Exposure to ionizing radiation may cause a reduction of the number of T lymphocytes and thereby affect cell bound immune mechanisms (3 9) In the case to be presented extremely low counts of circulating T lymphocytes were found and a considerable proportion of these had chromosomal aberrations The possible association between the abnormalities of the T cell population and the evolution of the clinical symptoms is therefore considered

CASE REPORT

The patient a 60 year-old man was admitted in Aug 1973 with complaints of fatigue and shortness of breath Past history revealed hospitalization for operation of a benign cerebral tumour in 1939 For diagnosis 8 ml of thorotrast had been used for carotid angiography In 1960 a routine X ray of the chest showed signs of fibrosis of the lungs Sarcoidosis was suspected and the diagnosis was confirmed by lymph node biopsies

In the spring of 1973 he felt increasingly tired and again called at the hospital At this time he had a severe anaemia Laboratory and cytological studies (see below) revealed myelosclerosis and splenomegaly with myeloid metaplasia X ray studies showed small densities dispersed throughout the spleen The patient was treated with blood transfusions and was given oximetholone for a short period There was an increasing need for transfusions and in the summer of 1974 splenectomy was performed in an attempt to increase the interval between them

In Sept 1974 gradually increasing numbers of blast cells were noted in the peripheral blood In March 1975 he had massive pleural effusions containing large numbers of blast cells There was a gradual deterioration and the patient died in April 1975 with overt signs of acute myeloblastic leukaemia Myelofibrosis and a widespread infiltration of blast cells in various organs were found at autopsy

Laboratory studies

In the summer of 1973 the following studies were performed

Cytology Aspiration from the sternum and the iliac crest yielded mostly peripheral blood and only few bone marrow cells The erythroid cells had a normoblastic appearance Fine needle aspirates from the spleen were rich in cells The white and red pulp were represented in normal proportions A complete absence of germinoblasts was noted The red pulp areas were rich in cells 20–25%



Fig 1 Autoradiographic preparation of smear from the spleen showing the characteristic α tracks of thorium emission (×984)

of which were haemopoietic with a conspicious predominance of megakaryocytes and erythroblasts often with the appearance of megaloblasts Pulp cells were rich in phagocytozed material Plasma cells were present in normal numbers When especially sought for a few groups of epithelioid cells were found Thus the main abnormality in the spleen aspirates was a myeloid metaplasia

Whole body measurement of radioactivity performed by Y Naversten Institue of Radiophysics University of Lund showed a pattern typical of thorium emission The mass of radiation was emitted from the spleen–liver region but emission was also counted from the hip regions

Autoradiography was performed on spleen aspirates thin sections of lymph node tissue and on sections of the spleen obtained at splenectomy Smear preparations and sections were covered with liquid nuclear film emulsion (Ilford K 2) according to the dipping method After exposure at 4 C for one to three weeks the autoradiographs were developed in Kodak D 19 (5 min 18 C) briefly rinsed in distilled water and fixed in Kodak F 24 (6 min 18 C) They were stained through the film with acid hematoxylin In all preparations tracks characteristic of α-emission were observed (Fig 1)

Lymphocyte studies The number of peripheral blood lymphocytes was calculated from a WBC and a differential count A pronounced lymphocytopenia 1 0×10⁹/l was found (normal range 2 0+0 5 (S D)×10⁹/l)

Using highly purified lymphocyte suspensions (2) obtained from defibrinated venous blood the percentage of lymphocytes forming rosettes with sheep red cells was determined according to Jondal et al (6) on two occasions The percentage of rosette forming lymphocytes (T lymphocytes) was 10% and 11% respectively which is considerably below the normal value of 55±8% (S D) in our laboratory

Chromosome analyses were made on lymphocytes incubated with PHA for 72 h and stained by a trypsin Giemsa banding technique described elsewhere (7) A total of 82 cells were counted the chromosome count showed the following distribution

Chromosome number	43	44	45	46	47	Total
Number of cells	1	7	38	35	1	82

Thirty of these cells were analysed in detail The 46-chromosome cells had a normal karyotype whereas all cells analysed with deviating chromosome numbers had in addition to gains and losses of normal chromosomes 1–4 different conspicuous marker chromosomes

DISCUSSION

Signs of myelosclerosis including dry taps on bone marrow aspiration and a myeloid metaplasia in the enlarged spleen were found in our patient 34 years after administration of thorotrast Myelofibrosis was confirmed at autopsy Myelosclerosis is a rare disease and in most cases the pathogenesis is obscure The demonstration of thorium emission from the skeleton may be relevant for the development of myelofibrosis in our patient since similar conditions have been induced experimentally by irradiation of the bone marrow (10) We therefore feel justified in considering the possibility that the myelosclerosis may have been a long term consequence of thorium radiation in the bone marrow

Since a reduction of immunologically active cells may cause an impaired immune surveillance favouring tumour development (1) the pronounced deficiency in T lymphocytes may have been of some importance for the development of malignancy in our patient In the lymphocyte cultures distinctive chromosome abnormalities including different marker chromosomes were noted in a large fraction (57%) of the PHA induced mitoses It is generally believed that lymphocytes stimulated to mitosis by PHA are T-cells Our findings therefore indicate that part of the small T cell population was abnormal which further emphasizes that the cell mediated immunological capacity may have been seriously depressed

The signs of sarcoidosis observed about 20 years after the injection of thorotrast may have been coincidental However pulmonary granulomatous lesions including macrophages and multinucleated giant cells have been observed in rabbits 2–4 years after an i v injection of thorium (5) The finding of T lymphocytopenia in our patient may also have some bearing on the sarcoid changes since sub-

normal numbers of T cells are characteristic in patients with sarcoidosis (4) It is not known whether this abnormality precedes the development of sarcoidosis or is a consequence of the disease (4) It is therefore an interesting possibility that a deficiency in T-cells may in some way favour the development of the granulomatous lesions in sarcoidosis

The multiple symptoms developing in our patient after a long term exposure to thorotrast may be attributed to various biological effects of ionizing radiation Thus a destructive effect on proliferating haemopoietic cells may have caused myelofibrosis The mutagenic action of the irradiation may have favoured the development of a frankly malignant disease Moreover a severe depression of cell bound immunity may have some bearing on both the formation of sarcoid changes and the development of malignancy

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Treatment of Advanced Breast Cancer with Chemotherapeutics and Inhibition of Coagulation and Fibrinolysis

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ABSTRACT A case of advanced breast cancer with cerebral metastasis and pleurisy is reported in which irradiation and cytostatics had failed to retard progressive growth and spread of the tumour Adjuvant therapy with heparin combined with the fibrinolytic inhibitor tranexamic acid was followed by regression of the cerebral metastasis as well as the pleurisy When last seen one year later the patient was free from symptoms

Tumour growth has been compared to wound healing with the formation of a fibrin matrix in which fibroblasts proliferate followed by vascularization (14 15 20 24) As in tissue repair the fibrinolytic activity of the tumour (20) is of importance for the removal of residual fibrin (2) Adjuvant therapy of malignant tumours with heparin or warfarin has been reported (11 23) We thought that anticoagulant therapy to prevent fibrin deposition combined with antifibrinolytic therapy to prevent dissolution of fibrin already formed might more effectively interfere with and have a static effect on the tumour growth This paper reports a case of metastasizing breast cancer that responded favourably to adjuvant therapy with heparin and the fibrinolytic inhibitor tranexamic acid

CASE REPORT

The patient was a para I born in 1927 In 1967 she was operated upon for carcinoma of the right breast Metastases were found in the axillary lymph nodes Radiation therapy was given postoperatively She was free from symptoms until 1974 when osteolytic metastases and pleurisy were diagnosed Treatment with X ray castration and androgen as well as Thio-tepa intrapleurally had no noteworthy effect The condi[illegible]equired repeated thoracocentesis In the middle of Nov 1974 the patient was given radioactive Au Chemotherapy with 5-fluorouracil methotrexate cyclophosphamide and cortisone according to Canellos et al (5) was tried but without success

In the beginning of Dec 1974 the patient was admitted to the Department of Radiotherapy Malmo General Hospital with signs of growth of the tumour Left sided hemiplegia occurred A 99 Tc cerebral scintiscan and angiogram of the right carotid artery revealed two metastases (Fig 1) The patient was rated at 10 points on the Karnofsky scale The affected area of the brain was irradiated with cobalt besides which the patient was given β methasone (Betapred®) This treatment was followed by substantial abatement of the clinical symptoms Antineoplastic therapy was continued with prednimustine i e a prednisolone ester of chlorambucil and methotrexate with leucovorin rescue During this therapy however leukopenia and thrombocytopenia supervened Fibrin degradation products (FDP) in the serum were found in concentrations of 20–60 μg/ml There was no effect on the pleurisy which still required repeated thoracocentesis Neither a 99 Tc scintigram nor X ray of skeleton showed any further change

Chemotherapy was therefore discontinued and the patient was given heparin subcutaneously in a dose of 12 500 IU twice a day combined with the fibrinolytic inhibitor tranexamic acid (Cyklokapron®) 1 g three times a day This treatment had a dramatic effect on the pleurisy and made further thoracocentesis unnecessary At a check X ray two weeks later no pleural effusion could be demonstrated The serum no longer contained FDP After one month s treatment with heparin and tranexamic acid combined chemotherapy was repeated every two weeks (vincristine methotrexate leucovorin 5-fluorouracil) Neither leukopenia nor thrombocytopenia recurred

When last seen the patient was free from noteworthy clinical symptoms and was rated at 80 points on the Karnofsky scale X ray showed no signs of pleurisy Skeletal X ray showed osteosclerotic metastasis An impressive effect of the treatment was the finding of [illegible] al scintiscan (Fig 2)

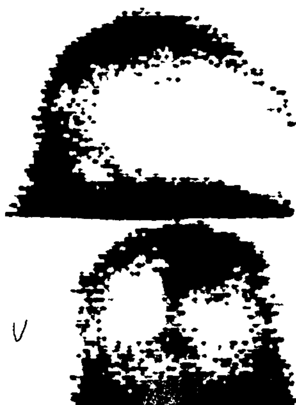


Fig 1 Cerebral scintiscan at the time of detection of the metastasis



Fig 2 Regression after treatment

COMMENT

The therapy thus resulted in regression of the cerebral metastasis and disappearance of the pleurisy It is debatable to what extent the encouraging results can be ascribed to the adjuvant anticoagulant and *antifibrinolytic therapy*

Cobalt radiation was followed by remission of the cerebral symptoms in agreement with observations by others (7 10 19 25) But survival is reported to be short usually about three months rarely more than a year Our patient has now been free from symptoms for 18 months since detection of her cerebral metastasis The cytostatic drugs given enter the cerebral tissue in only low concentrations The anticoagulant therapy might have facilitated the penetration of the drugs into the tumour mass as suggested by Elias et al (11)

There are several reports on the coagulative properties of malignant tumours (4 8 12 15 16 17 21) Recently Gordon et al (13) reported the presence of a cancer procoagulant in malignant tissue which was characterized as a serine protease that did not require factor VII for its procoagulant activity but was capable of initiating coagulation by direct activation of factor X This supports the opinion that certain coagulative properties of tumours are necessary for the deposition of fibrin *facilitating* the proliferation of vessels

The fibrinolytic properties of tumours are well known (20) Tumours in culture release activators initiating the fibrinolytic system (3 9) Rifkin et al (18) reported release of fibrinolytic enzymes by mammalian fibroblasts transformed by DNA or RNA viruses or by chemically or virally induced mammary carcinomas as well as by human malignant tumours in culture Normal fibroblasts and cultures of other normal tissue release little or no fibrinolytic enzymes Christman and Acs (6) found neoplastic cells whether transformed by oncogenic viruses or by chemical agents to release a plasminogen activator not released by normal cells before transformation Åstedt and Holm

berg (1) reported a plasminogen activator produced by ovarian carcinoma in culture to be immunologically identical to urokinase This indicates that also certain fibrinolytic properties are necessary for tumour growth probably for the removal of residual fibrin

The significance of coagulative and fibrinolytic properties of malignant tumours in the production of ascitic and pleural exudate is obvious from the large amounts of FDP in these fluids (22) Administration of cytostatics failed to have any effect on the pleurisy and repeated thoracocentesis was necessary Heparin and tranexamic acid soon eliminated the pleural effusion which has not recurred since We have observed a similar favourable effect in patients with malignant ascitic fluid especially in cases of mesothelioma and ovarian cancer This suggests a direct effect of the anticoagulant and antifibrinolytic therapy on the regression of the pleural exudate

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BOOK REVIEWS

Therapie Fibel der inneren Medizin für Klinik und Praxis Von Prof Dr Dr S Moeschlin Solothurn 5 neu bearbeitete und erweiterte Auflage xxiv 716 Seiten DM 60 — (ISBN 3133785052) Georg Thieme Verlag Stuttgart 1976

Sven Moeschlin has published the 5th edition of his thera peutic manual of internal medicine The first appeared in 1961 Since the 4th edition in 1974 the new one has to a large extent been rewritten and brought up to modern standards This monograph is a handbook rather than a manual The content covers not only internal medicine but also cardiology endocrinology hematology infectious diseases nephrology neurology oncology and tropical diseases

Internal medicine has in its development reached a cross road It has become too large a topic for anyone to master completely The huge mansion has split into cottages where subspecialists are happy to rule their own departments Moeschlin one of the last medical poly histors strives to command the whole of internal medicine He succeeds

In the foreword he states concerning therapy that the choice lies between let well alone and too much Here at least there is plenty a therapeutic encyclopedia and a survey of internal medicine full of facts and hard to digest in an afternoon Therapeutic advice is combined with diagnostic hints all based on the author s clinical [illegible] and command of literature As a matter of fact [illegible] is this type of programmed clinical manual which is now [illegible]der debate in Sweden In order to make treatment more equal such programs are now under production Compared with the Swedish trends Moeschlin s manual offers a much richer list of preparations This reflects the base for his choice clinical experience while in Sweden the base is theoretical pharmacology and pharmacy Moeschlin s book contains very little pharmacology which is appropriate for a manual The arrangement is masterly and good illustrations and tables alleviate the reading The German language is light and enjoyable

The author gives his advice very decisively Alternative treatments are always given but very little discussion of the choice between them There is much good advice on precautions against side-effects and difficulties

In its principal parts treatment in internal medicine follows internationally adopted rules Where details are concerned there are however national differences This complicates comparisons between different schools of treatment Moeschlin follows the old German style and gives a complete list of all treatment possibilities but does not always compare their value Many subjects are bound to be debatable Doctors also are great individualists in their treatment

I am for instance astonished to find so many anal gesics containing metamizol recommended Baralgin® Novalgin® Buscopan comp ® and even amidopyrine In Sweden they were removed long ago because of the risk of agranulocytosis Moeschlin himself a clinical pioneer of agranulocytosis has no objections or reservations about their use

As far as antibiotics are concerned, he recommends the use of combinations of preparations for lengthy prophy lactic courses This should prevent resistance and give a more powerful effect He is greatly in favour of the combination of penicillin and streptomycin The latter drug is practically out of use in Sweden not only be cause of the risk of damage to N VIII but also the risk of sensibilization of the personnel handling it

Personally I will not subscribe to the use of nicetamide Complamin® and Ronicol® as vasodilators and Aldo met® and Prednisone® in the treatment of carcinoid flush Dextran (Macrodex®) should not be used in bleeding peptic ulcer as it is an antithrombotic anticoagulant The combination of Enterovioform® Mexaform® and ampi cillin in acute gastroenteritis seems useless I hesitate about the prophylactic use of penicillin during 5 years after rheumatic fever and one year after acute nephritis

Treatment of essential hypertension is a debatable sub ject and the preferred lines of treatment change every other year At present the β blocking agents are very much to the fore and are often used as first-choice drug The author recommends them mainly in hypertensive crises Reserpin no longer has any advocates here because of the risk of suicide In the treatment of thrombosis the author abstains from the early use of heparin in com bination with dicoumarol which we use as a standard pro cedure where thrombolytic therapy is not used The use of Konakion® in hemoptysis is of no avail Trasylol® is out of date and has no documented effects either in fibrinolysis or acute pancreatitis where peritoneal dialysis is the first choice of treatment There is no risk of rethrombosis if dicoumarol is omitted abruptly This drug has a slow action

These are a few marginal notes which do not sub tract from the practical value of the book to all physi cians and also surgeons The content of the book is so overwhelmingly large that a detailed scrutiny is impossible Everyone can choose his likes and dislikes but no one can blindly endorse all the author s opinions On the whole it is a collection of therapeutical advice not to be found anywhere else

Internal medicine in central Europe has by tradition a hierarchic structure In spite of the firm guidance in this therapeutic manual it offers a better chance of free treatment to the physician than many other systems

Moeschlin s manual is too heavy to carry in the pocket Its impact on the practice of medicine is also heavy It should be found on the desk of every physician practising internal medicine It is strongly recommended

Sven Erik Bjorkman Malmo Sweden

British Medical Bulletin Haemoglobin Structure function and synthesis 100 pages £3 50 British Council 65 Davies Street London W1Y 2 AA 1976

Some twenty years ago it was generally accepted that Scandinavian clinicians could only have a platonic interest in the hemoglobinopathies because we never met them in real life This has now changed markedly for two reasons The fascinating progress in molecular biology as applied to bedside medicine has in great part been made in two fields hemoglobin and immunoglobulins Our knowledge regarding the hemoglobin molecule is so intimate that we know the different deletorious clinical results of an exchange of one amino acid in some of the strategic positions The other reason is that we have realized—as might be expected—that also in our countries mutations of hemoglobin synthesis may be found and be linked to special disease pictures

This number of the British Medical Bulletin gives a superb review of British work on these problems Ingram who made the first real breakthrough with his fingerprinting of the different polypeptides is British The large group at the Cavendish laboratories in Cambridge – with Perutz in chemistry and Lehmann in genetics and clinical medicine as leaders—as well as many other groups in Great Britain have been great roadbuilders in these fields No *wonder that the volume is excellent*

I should like only to pick out a few questions treated in this volume The fact that the binding capacity of the hemoglobin decides erythropoetin production and therefore the levels of erythrocytes in the blood has been illustrated by recent clinical studies If the hemoglobin binds O_2 too firmly this leads to tissue anoxia and *secondary increase in erythropoetin production and* polyglobulia just as it happens at high altitudes For some years it has been clear that certain exchanges in amino acids in strategic points of the molecule are the explanation of hereditary and early polyglobulia There are now at least eleven such substitutions all in the same region of the molecule illustrating the fact that a certain microstructure *is necessary for the normal function of the hemo*globin as an oxygen carrier This knowledge is important because it shows the close contact between the most sophisticated biochemistry and bedside medicine It should also persuade the doctor that the condition should not be treated

Hemoglobin Malmo is one of these mutations It is interesting *that the same amino acid in the β-chain* (aspartic acid β 99) has been substituted by four different amino acids in four hemoglobin variants with the same clinical results whereas Malmo hemoglobin is a substitution in β 97 a next neighbour It may be said that a certain number of amino acid substitutions in the region of contact between α_1 1 and β 1 chains has such effects regarding the affinity for oxygen

The latest condition discovered in this chapter of molecular hematology is perhaps still more interesting It has been found that certain anemias might possibly be explained by a reverse effect If the hemoglobin gives off oxygen too easily the tissues get oxygenated in excess and erythropoetin production is decreased This is a completely new potential cause of chronic anemia So far it has only been found in very few cases but it may well be assumed that a number of conditions with stable moderate anemia could be explained by this mechanism The technique that is necessary in order to detect such a pathological hemoglobin is quite complicated however and the number of such diagnoses will probably remain very moderate until easier techniques have been described

We do not know yet how many malformations of the hemoglobin molecule may occur but it is interesting that a recent study with systematic hemoglobin electrophoresis in 3 000 persons in Norway disclosed one abnormal hemoglobin that was obviously a mutant even if it did not cause any clinical symptoms This is hemoglobin Sogn that was described in 1968 It is slightly unstable and faint signs of hyperhemolysis were detected on careful examination An unstable hemoglobin causing hemolytic anemia is hemoglobin Borås (Sweden) An exchange in the same position was already known as Santa Ana from California The amino acid substitutions are different Nothing is known about the real incidence of hemoglobins with amino acid substitutions in functionally more or less unimportant sites It is probable however that screening procedures possibly also with isoelectric focusing and measurement of oxygen affinities with simpler techniques will bring many new varieties to light also in our part of the world

An enormous screening for abnormal hemoglobins in a quarter million people was recently performed in an almost entirely (97 5%) black population in Texas USA Hemoglobin S and C dominated completely (8 6 viz 2 4%) Places 5 and 6 were taken by two hemoglobins with two substitutions of the same amino acid (β 121) One of these is called Los Angeles or Punjab and this is one of the few hemoglobinopathies that have been found in Scandinavia It has occurred in Norway (Sogn) and is also known from Sweden Four new hemoglobins were found

I think that these data show that the stability of the hemoglobin molecular structure is not what we had imagined a decade ago

The most fascinating new developments in hemoglobin research described in this volume of the British Medical Bulletin is to my mind connected with the transition from the sequencing of amino acids in the globin molecules to a detailed study of the templates that form this protein It is now possible to describe such mutations by exactly stating the changes in nucleotide triplets that are the basis for a faulty assembly of the polypeptide chains in the hemoglobin molecule From recent work it seems probable that some such nucleotide changes result in nonsense sequences thus possibly explaining why α or β chains are not produced in the thalassemias

Other chapters treat the effect of DPG a substance that has great importance for the understanding of anemia unstable hemoglobins as cause of hyperhemolysis and of course problems of sickling and thalassemia that are farther away from Scandinavia but still interesting as examples

This is truly an exciting book for all scientists interested in the basic mechanisms at work in protein synthesis The introductory remarks of the grand old man in

globin chemistry M F Perutz may be quoted as a summary Hemoglobin was the first protein to be cristallized already in 1849 by a German biochemist Its molecular weight was the first among the proteins to be determined and Perutz quotes Adair in this connection The reviewer would like to add the name of The Sved berg who studied these molecules in the ultracentrifuge invented by him The first protein to be synthesized in vitro in a cell free system derived from an eukaryocytic organism was hemoglobin This proved that the mecha nisms is the same as in E coli The last advance was the isolation of messenger RNA for globin and the deter mination of its nucleotide base sequence as described in this volume It was also in hemoglobin that Ingram discovered the fundamental fact that replacement of one singular amino acid in the long polypeptide chain con tained in one half molecule hemoglobin is the basis of an inborn error of metabolism This was truly the basis of clinical medicine on the molecular level

The volume is intellectually fascinating It may be strongly recommended to all physicians and non medical biologists who want to learn about recent advances on the molecular level

Jan G Waldenstrom

Acta Med Scand 201 497–508 1977

MODERN MEDICAL HISTORY

Von Willebrand's Disease—Fifty Years Old

Inga Marie Nilsson

From the Coagulation Laboratory Malmo General Hospital Malmo Sweden

It is now exactly 50 years since Professor Erik von Willebrand in Helsinki (Fig 1) published his first paper on an inheritable bleeding disease that he had observed in several members of a family from Foglo on the islands of Åland in the Gulf of Bothnia (Fig 2) The title of his paper was Hereditar pseudohemofili , and it was published in the proceedings of the Finnish Society of Medicine in 1926 The article was written in Swedish but with a German summary Von Willebrand s first case was seen in a girl Hjordis When first examined she was 5 years old (Fig 3) She belonged to a sibship of 12 children all but two of whom had had bleeding symptoms Four of the sisters had died at 2–4 years of age from uncontrollable bleeding from the nose wounds and intestines Both parents had when young had troublesome nose bleedings Von Willebrand then studied 66 family members and found 23 of them to have symptoms of the same haemorrhagic diathesis as Hjordis (72) (Fig 4) The family, now the well known Åland family has since been studied more extensively and is still the one with by far the largest number of members with known von Willebrand s disease

In his publication von Willebrand gave a detailed description of the clinical picture of the disease The dominating symptoms consisted of nose bleeding bleeding from the gums and after tooth extraction bleeding from the female genital tract and bleeding from trivial wounds Hjordis died from her fourth menstruation Von Willebrand stressed that joint bleedings common in haemophilia were relatively rare A prolonged bleeding time despite a normal platelet count was its most important characteristic He found the coagulation time to be normal Clot retraction was studied only in Hjordis and was found to be normal The Rumpel Leede test was positive He felt that blood transfusions were useful not only for replacing blood loss but also for controlling bleeding The inheritance of the disease was believed to be autosomal dominant Von Willebrand distinguished the disease from anaphylactoid purpura and thrombocytopenic purpura as well as from the hereditary haemorrhagic thrombasthenia described by Glanzmann (21) He concluded that the condition was a previously unknown form of haemophilia that affected both sexes and he called the disease hereditary pseudohaemophilia A prolonged bleeding time was its most important characteristic Von Willebrand also discussed the pathogenesis of the condition and found that the bleedings could best be explained by the combined effect of a functional disorder of the platelets and a systemic lesion of the vessel walls This first description of the disease is really impressive In 1928 the clinical picture was reported independently by four Americans including Minot (47)

In 1933 von Willebrand began to collaborate with a German physician Rudolf Jurgens who was to investigate the cause of the faulty haemostasis (73 74) In the following years Jurgens repeatedly visited Åland and tried out every new available coagulation method on these patients Unfortunately these investigations did not result in any clarification of the disease Jurgens therefore ultimately felt that the bleeding tendency in this disease was due to some impairment of platelet function including platelet factor 3 deficiency (38) This resulted in the disease being called von Willebrand Jurgens thrombopathy Further cases were discovered in Åland Genetic studies by Lehmann (44) and Eriksson et al (19) have shown that practically all the cases in

Fig 1 Erik Adolf von Willebrand

Åland can be traced to von Willebrand s original Foglö family and that it is inherited as an autosomal dominant characteristic In the last five generations of this family as many as 132 members are known to have been affected My teacher the late Professor Erik Jorpes came from Kokar in Åland (Fig 2) He several relatives with von Willebrand s disease

From the middle of the 1930s until the middle of the 1950s several cases of hereditary haemorrhagic diseases with prolonged bleeding time and normal platelet count were reported from Europe and the USA In 1956 Buchanan and Leavell (14) published a large compilation of such cases under the title of Pseudohemophilia Clot retraction was said to be normal in the vast majority of these cases Most of them were probably examples of von Willebrand s disease

In the beginning of the 1950s improved methods were devised for determination of the antihaemophilic factor, factor VIII In 1953 Alexander and Goldstein (3) described 2 cases with a combined defect of haemostasis namely a prolonged bleeding time and decreased factor VIII activity Similar cases were later observed by a number of other authors such as Larrieu and Soulier (43) Quick and Hussey (56) van Creveld et al (17) Singer and Ramot (62) and Schulman et al (58) Most authors thought that the prolonged bleeding time was due to some defect in the vessel wall Schulman therefore called this bleeding syndrome vascular haemophilia

In 1956 and 1957 together with Margareta Blomback and Irene von Francken I described 13 members of 10 families in Sweden who had a congenital haemorrhagic diathesis characterized by factor VIII deficiency and prolonged bleeding time (50, 51) Genetic studies showed that the gene which caused factor VIII deficiency was autosomal and dominant but with varying expressivity All these cases had previously been regarded as instances of thrombopathy At that time Birger and Margareta Blomback in Stockholm were working on a method for the purification of fibrinogen by treatment of Cohn s fraction I with 1 M glycine solution (5) Since we found that the factor VIII activity of the starting plasma could be almost quantitatively recovered in fraction I–0 this fraction was prepared under sterile conditions and its effect was tried on patients with this bleeding disease After the injection of fraction I–0 bleeding stopped and the factor VIII deficiency disappeared as did the prolongation of the bleeding time This was interesting since it argued against a disorder of the capillary wall as the cause of the prolonged bleeding time and suggested that it was due to a deficiency of a plasma factor that could be recovered in fraction I–0 from normal plasma

The clinical symptoms and the mode of inheritance of our cases closely resembled those in von

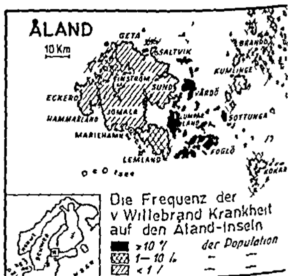


Fig 2 The islands of Åland in the Gulf of Bothnia (From Eriksson A W Acta Genet Med Gemellol (Roma) 10 157 1961)

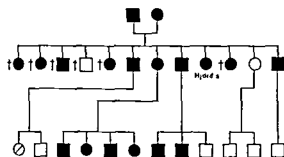


Fig 3 It was this family in which five girls died from uncontrollable bleeding between the ages of 2 and 14 years that prompted E A von Willebrand to start his investigation on Åland

Willebrand s disease but not the laboratory findings In his investigation of von Willebrand s disease on Åland Jurgens found a normal factor VIII content but an abnormal platelet function which could not be demonstrated in our patients

In June 1957 I went to Åland with Margareta Blomback Erik Jorpes and a young student Arne Johansson (52) Erik Jorpes was really enthusiastic and very keen on visiting his native island and he arranged everything for us in the little city of Mariehamn I feel I have my best memories of him from that trip We took with us the equipment of almost a whole coagulation laboratory with cold centrifuges water baths and so on The electric mains in Mariehamn could not stand the load and the young student who soon got the nick name platelet Johan had to fly once or twice every day between Mariehamn and Stockholm for centrifugation of the plasma samples for platelet preparation We studied 15 patients belonging to the Åland family including some who had been examined 25–30 years previously by von Willebrand himself Margareta Blombäck and I were very busy with blood sampling and all the coagulation assays Erik Jorpes felt himself useless and as he could not stand being idle he began to wash all our tubes and pipettes He did it perfectly and he enjoyed it We found the factor VIII activity to be deficient and platelet function as well as platelet factor 3 to be normal One of the patients in Åland was treated with fraction I–0 which normalized the factor VIII level and the bleeding time

These investigations thus showed that the disease which had been described in several quarters of the world as being characterized by a reduced factor VIII level and prolonged bleeding time was identical with that described by v nd

The finding that it was possible to correct the prolonged bleeding time in von Willebrand s disease by injection of human fraction I–0 opened up a new approach to the investigation of the pathogenesis Fraction I–0 prepared from haemophilia A plasma also corrected the bleeding time (49) (Table I) Fibrinogen had no effect Since it made no difference whether fraction I–0 was prepared from platelet poor or platelet rich plasma the correction of the abnormal bleeding times in von Willebrand patients obviously had to be due to a plasma factor not necessarily associated with the factor VIII activity This plasma factor became subsequently known as the von Willebrand factor

We observed that the factor VIII activity successively increased during the first 24 hours after the injection of fraction I–0 to patients with von Willebrand s disease in contrast to what was seen in haemophilia (Fig 5) Fraction I–0 prepared from plasma from patients with severe haemophilia A not only corrected the bleeding time but also generated factor VIII activity The maximum rise in factor VIII did not occur immediately at the earliest 5–8 hours after the transfusion It was therefore concluded that the above mentioned von Willebrand factor not only affected the bleeding time but also stimulated the production of factor VIII activity The results obtained on administration of different sorts of plasma and plasma fractions in von Willebrand s disease have since been widely confirmed by several researchers (16) Anyhow the von Willebrand factor appeared to be closely related to factor VIII and it occurred in factor VIII rich plasma concentrates

It was at first not known how a plasma factor

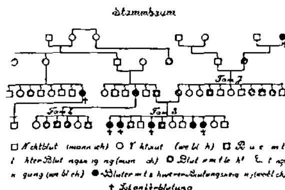


Fig 4 Pedigree of the Åland family (From von W brand E A Über hereditäre Pseudohamophilie Med Scand 76 521 1931)

Table I *Effect of various preparations on patients with von Willebrand's disease*

Preparation	Effect of preparation		
	Increase in AHF content	Normalization of bleeding time	Increase in fibrinogen content
I-0 from normal plasma	+	+	+
I-0 from haemophilia A plasma	+	+	+
I-2-F (fibrinogen separated from normal I-0)	−	−	+
Purified fibrinogen from bank blood	−	−	+
Platelet suspension	−	−	−
Fresh plasma (silicon technique)	+	+	−

could affect primary haemostasis and shorten the bleeding time The basis of primary haemostasis is the formation of a platelet plug Borchgrevink (9) found decreased platelet adhesiveness in vivo in patients with von Willebrand s disease by comparing the platelet count in venous blood and in blood from a capillary lesion A reliable in vitro test for measuring the interaction between platelets and a glass surface was devised by Hellem (24) Hellem s original method which used citrated blood did not distinguish normal persons from those with von Willebrand s disease Salzman (57) used native blood

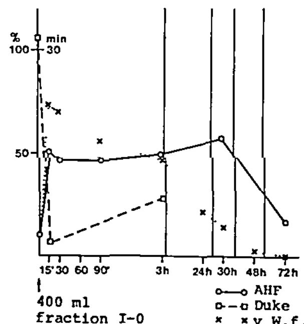


Fig 5 The typical response to infusion of factor VIII concentrate in von Willebrand s disease v W f =factor VIII related antigen AHF=factor VIII activity (From Nilsson I M and Holmberg L In Transfusion and immunology (ed E Ikkala and A Nykanen) p 235 Vammalan Kirjapaino Vammala 1975)

and a quicker flow and could then demonstrate a decreased platelet adhesiveness to glass in von Willebrand s disease Modifications of Salzman s method all with a rapid blood flow have also proved diagnostically useful

The reduced platelet adhesiveness in von Willebrand s disease like the bleeding time can be normalized by infusion of fresh platelet poor plasma as shown by Salzman Such normalization can be demonstrated in vitro by mixing the patient s blood with normal plasma haemophilia A plasma or cryoprecipitate This was first demonstrated by Meyer and Larrieu (46) The factor causing such normalization is missing in von Willebrand plasma

Further knowledge about the pathogenesis of von Willebrand s disease has been obtained in recent years since the advent of highly purified factor VIII (AHF) The AHF as isolated from plasma is a very large macroglobulin In 1971 Stites et al (63) and Zimmerman et al (75) raised monospecific precipitating rabbit antisera against this globulin Immunological analysis showed that patients with haemophilia A have a cross reacting but inactive protein in the plasma while the majority of patients with von Willebrand s disease have a low content of this so-called factor VIII related antigen The chromatographic pattern of normal and haemophilia cryoprecipitate on Sepharose 6B according to van Mourik and Mochtar (48) shows a typical protein peak corresponding to the factor VIII related plasma protein in the void volume No such peak is seen when the starting material consists of cryoprecipitate from a patient with severe von Willebrand's disease This has been shown by Bouma et al (13) and by Holmberg (25)

Bouma et al (12) showed that the factor VIII related high molecular weight protein is responsible for enabling normal and haemophilic plasma to

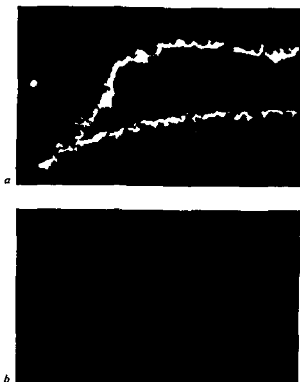


Fig 6 Factor VIII antigen in the vessel wall of *(a)* normal person *(b)* patient with severe von Willebrand s disease (× 1 600)

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normalize the reduced platelet adhesiveness in von Willebrand s disease This protein therefore possesses so-called von Willebrand factor activity

Patients with von Willebrand s disease have a defect in primary haemostasis with prolonged bleeding time and defect platelet adhesiveness Several workers have shown that the platelets of patients with von Willebrand s disease are normal in respect of aggregation and their release function (18 52 69 76) The molecular mechanism underlying a supposed interaction between factor VIII related protein and platelets in primary haemostasis is still unknown Some interesting observations have however been made Purified bovine and porcine high molecular weight AHF related proteins aggregate human platelets in vitro (20) An important observation was made in von Willebrand s disease in 1971 when Howard and Firkin (32) found that the antibiotic ristocetin failed to aggregate platelets in von Willebrand plasma A factor present in both normal and haemophilic plasma is necessary for the reaction and this ristocetin co-factor like factor VIII related antigen and factor VIII activity appears in the void volume when plasma is chromatographed on agarose columns Like other abnormalities in platelet behaviour in von Willebrand s disease a defective platelet response to ristocetin can be corrected by normal plasma Numerous endeavours have therefore been made to use ristocetin induced platelet aggregation as a diagnostic and research tool in the study of plasma components responsible for the haemostatic defect in von Willebrand s disease Several methods for determining plasma ristocetin co-factor have been described Kattlove and Gomez (39) have suggested that the ristocetin is bound to the platelet membrane and that factor VIII antigen forms a bridge between the ristocetin molecules causing aggregation Jenkins et al (37) believe that ristocetin acts on factor VIII related antigen adsorbed on platelet surface leading to exposure of active sites necessary for aggregation

Factor VIII related antigen has been identified in endothelial cells in tissues throughout the body with the aid of fluorescent conjugated antisera (7 23 34) Jaffe et al (35 36) have also shown that cultured human endothelial cells synthesize and release this protein The protein has also been demonstrated in platelets We have investigated factor VIII related antigen in the vessel walls of patients with von Willebrand s disease and haemophilia A (26) Biopsy specimens of superficial veins were obtained from 5 patients with von Willebrand s disease and from 3 with haemophilia A and studied for factor VIII antigen by means of a specific FITC-conjugated factor VIII antibody In all the 15 healthy men who served as controls as well as in the patients with haemophilia A factor VIII antigen was demonstrated in the vascular intima (Fig 6) Three patients with severe von Willebrand s disease with no factor VIII antigen in the plasma had no antigen in the intima either Even after infusion of factor VIII concentrate in these patients no factor VIII fluorescence was seen in the vessels Therefore infused factor VIII does not seem to adhere to endothelial surfaces to any notable extent (45)

As mentioned above factor VIII related antigen has been found in normal platelets As far as patients with von Willebrand s disease are concerned opinions differ Shearn et al (59) and Bouma et al (11) have found factor VIII related antigen in platelets of patients with von Willebrand s disease but Howard et al (33) and Coller et al (15) found no factor VIII in platelets from their patients In 10 patients with severe von Willebrand s disease we have not been able to demonstrate factor VIII related antigen in their platelets by immunochemical methods Not even after infusion of factor VIII could we demonstrate any factor VIII protein in the platelets while in patients with mild von Willebrand s disease factor VIII protein could be demonstrated

Much indicates that factor VIII related antigen or the von Willebrand factor is necessary for the adhesion of platelets to the subendothelium Which localization of the factor in plasma in platelets or in the vessel endothelium, is of greatest importance for this function is difficult to say Since all the defects in the disease can be controlled by infusion of plasma or plasma concentrates it is possible that the plasma factor is the most important

What is the structure of the factor VIII protein? Structurally it is a macroglobulin with a very high molecular weight about 2 millions It is composed of very similar subunits Does it consist of two components one possessing the factor VIII activity and the other the von Willebrand factor activity or does it consist of a single but bifunctional molecule? There is evidence in support of both theories but neither has been definitely proven Quite recently Switzer and McKee (65) published a study on factor VIII Their results supported the exis

Table II *Frequency (%) of remarkable bleeding in the Swedish series (264 cases) of von Willebrand's disease (Silwer (60))*

Nose bleeding	62.5
Meno-metrorrhagia	60 1*
Post-extraction haemorrhage	51 5
Ecchymoses and haematomas	49 2
Bleeding from trivial sores and wounds	36 0
Gingival bleeding	34 8
Postoperative bleeding	28 0
Bleeding at delivery	23 3
Gastrointestinal bleeding	14 0
Traumatic oral and lip bleeding	11 7
Petechiae	11 5
Joint bleeding	8 3
Haematuria	6 8
Ovarian bleeding	6 8
Bleeding from tonsils	6 1
Bleeding during shedding of teeth	4 9
Bleeding at abortion	3 8
Intramuscular deep subcutaneous or submucous bleeding	2 7
Bleeding from ears	3 0
Haemoptysis	1 9

* Calculated for females above 15 years

tence of a covalently linked subunit structure for factor VIII and suggest that the molecule has both coagulant and von Willebrand factor activity We feel that if the molecule is to have von Willebrand factor activity it must have a very special quaternary structure Autosomal genes are responsible for polymerization of the chains to a large molecule with the special configuration In von Willebrand s disease the defect may consist of impairment of the ability to form high molecular weight aggregates from the building stones—the protein chains

In this connection the transfusion response in von Willebrand s disease has to be considered Administration of factor VIII concentrate to a patient with severe von Willebrand s disease has a number of effects (Fig 5) It will shorten the bleeding time and elicit an increase in factor VIII activity out of proportion to the activity of the concentrate But this is not accompanied by any such increase in the amount of antigenic protein which instead decreases successively (4 29) This means that the factor VIII activity may occur in a part of the molecule which does not react to the antiserum with any demonstrable precipitation

There are four major classes of activities related to the factor VIII protein complex 1) it acts in intrinsic plasma coagulation—factor VIII coagulant activity (VIII C) 2) it is precipitated by specific rabbit antiserum—factor VIII related antigen (VIIIR AG) 3) it probably interacts with platelets in primary haemostasis (20 32 68) and it is necessary to keep the bleeding time and the in vitro platelet adhesiveness normal (13)—von Willebrand factor activity—and 4) it aggregates normal and von Willebrand platelets in the presence of ristocetin (31 32 71)—ristocetin co-factor activity (VIII Rcof)

In the classical type of von Willebrand s disease there is a quantitative defect of the factor VIII protein complex and all these activities are deficient

In recent years it has gradually been realized that von Willebrand s disease is not a uniform disease and several genetic variants have been recognized (see below)

Clinical aspects of von Willebrand's disease

Von Willebrand s disease seems to be one of the most common haemorrhagic diatheses In 1975 there were 785 known cases of von Willebrand's disease in Sweden This means an incidence of 1 in 10000 inhabitants The corresponding figure for haemophilia in Sweden is 1 in 10000 men i e barely 1 in 20000 of the population as a whole However mild cases may remain undiagnosed

Most authors agree that the disease is inherited by an autosomal and dominant gene Silwer (60) has recently surveyed all known cases of von Willebrand s disease in Sweden He found the disease in relatives of probands in 88% of the families examined The penetrance as judged from examination of the parents was 73–90% The expressivity varied widely often within the same family But analysis of variance showed that the expression regarding the factor VIII content was much more uniform in the sibships than between unrelated patients The disease was more common among females than among males This is probably because women with von Willebrand s disease so often have menorrhagia which leads to detection of the disease He estimated the risk of a child of an affected person inheriting the disease to be 40% or less The risk of the disease being severe was however regarded as 5% or less

The clinical picture of the disease has been described in detail by von Willebrand himself and later by several other workers Silwer in his monograph of Swedish cases has given a detailed description as well as the frequency of the bleeding symptoms in severe and mild von Willebrand s disease (Table II) In severe von Willebrand s disease the bleedings

may be life threatening while in mild cases they may be so mild that the disease remains concealed

Nose bleeding is the most common symptom of von Willebrand s disease and occurs in 60–80% of affected patients (60) Bleeding from the oral cavity is also fairly common especially in children In contrast with what is seen in haemophilia deep subcutaneous and intramuscular haematomas are uncommon in von Willebrand s disease Very typical however are prolonged bleedings from trivial wounds

The most prominent bleeding symptom in women with von Willebrand s disease is probably menorrhagia Menstrual disorders occur in some 50–75% of such women The symptom is most severe during the first few years after menarche and has occasionally been life threatening and required hysterectomy or roentgen castration Menorrhagia is also often a difficult problem in von Willebrand s disease characterized as mild by laboratory tests Another type of bleeding from the female genital tract which has not received much attention is corpus luteum bleeding Typical cases are characterized by intermenstrual more or less severe abdominal pain and often by a low Hb because of intra abdominal bleeding

Delivery in women with von Willebrand s disease is often though not always associated with heavy blood loss In most women with von Willebrand s disease the AHF level rises during pregnancy which lessens the risk of bleeding The AHF however does not always rise and especially not in women with an initially very low level Even if no bleeding occurs at parturition the patient must be observed for at least a week post partum because the AHF level falls after delivery

In most published series of von Willebrand s disease the frequency of gastrointestinal bleeding is relatively low usually 10–15% It is however striking that when such bleeding occurs it is usually very severe

Joint bleedings are not very common in von Willebrand s disease in contrast to haemophilia but do occur in patients with severe disease In a Swedish series of 32 cases of severe von Willebrand s disease half of the patients had haemarthroses and seven chronic arthropathy (2)

Postoperative bleeding is a common and serious symptom in von Willebrand s disease Tooth extractions are often followed by prolonged oozing unless adequate precautions have been taken Operations in the region of the ear nose or throat such as tonsillectomy and adenoidectomy are often associated with prolonged serious bleeding This is true also of abdominal operations although it has sometimes been possible to perform an appendectomy without special precautions in patients with mild von Willebrand s disease In all operations the bleedings may increase or recur several days or even weeks after the operation

Diagnosis of von Willebrand's disease

Now after the discovery of several variants or types of the disease the differential diagnosis of von Willebrand s disease is not easy The classical type of von Willebrand s disease in which there is a quantitative defect of the factor VIII protein complex will be considered first The manifestations of bleeding like the laboratory findings in von Willebrand s disease tend to vary from time to time The symptoms often decrease in severity with increasing age The diagnosis of von Willebrand s disease must therefore be made jointly on the basis of the patient s history and the results of several laboratory tests

The characteristic findings in classical von Willebrand s disease are 1) Positive history of bleeding 2) positive heredity 3) prolonged bleeding time 4) decreased F VIII activity 5) decreased platelet adhesiveness according to Salzman and/or Bowie 6) decreased F VIII related antigen 7) decreased ristocetin co factor activity 8) retrograde increase of F VIII activity after infusion of F VIII or plasma We also think it is possible to diagnose von Willebrand s disease in those cases where (*a*) relatives have not been examined or affected relatives are unknown but the patient meets the requirements given under the other points and (*b*) typical disease in the family but not all findings under the other points are positive

I would stress that the above mentioned variation in the laboratory values must be borne in mind In patients with von Willebrand s disease as in normals the factor VIII activity fluctuates widely This applies particularly to mild and moderate cases of von Willebrand s disease The factor VIII activity also increases with increasing age Many conditions raise the level of factor VIII antigen There are two mechanisms which increase factor VIII antigen a fast one associated with physical stress and exercise or with adrenalin or vasopressin infusion and a slower one correlated to tissue break

Table III *Variants of von Willebrand's disease*

1	Classical (Åland)		2	VIII C	—→+	3	VIII C	+
	VIII C	–		VIIIR AG	+		VIIIR AG	–
	VIIIR AG	–		VIII WF (BT)	–		VIII WF (BT)	–
	VIII WF (BT)	–		VIII Rcof	–		VIII Rcof	(+)
	VIII Rcof	–						
	Cross imm normal			Cross imm normal			Gel filtration abnormal	
	Cross imm abnormal mobility			Cross imm *abnormal* mobility			Holmberg et al 1974	
				Firkin et al 1973				
				Kernoff et al 1974				
				Peake et al 1974				
				Stableforth et al 1974				
				Holmberg 1974				
				Gralnick et al 1975				
4	VIII C	–	5	VIII C	–			
	VIIIR AG	+		VIIIR AG	–			
	VIII WF	–		VIII WF (BT)	+			
	VIII Rcof	+		VIII Rcof	—→+			
	X-chromosomal (?)			Thomson et al 1974				
				Bloom et al 1974				
	Holmberg & Nilsson 1972			Veltkamp & v Tilburg 1974				
				Autosomal haemophilia				

– = low level or prolonged BT + = normal level and normal BT VIII C = factor VIII clotting activity, VIIIR AG = factor VIII related antigen VIII WF (BT) = bleeding time and platelet adhesiveness

down growth and repair Also in von Willebrand s disease factor VIII antigen can increase in various diseases associated with tissue damage such as cancer infections etc and in pregnancy (30) If due attention is given to this determination of factor VIII related antigen—most workers use the rocket method of Laurell—is very useful for the diagnosis

Usually Duke s or Ivy s method is used Duke s method is less sensitive and has often been reported to fluctuate In severe von Willebrand s disease Duke s bleeding time is however nearly always markedly prolonged The Ivy bleeding time and especially the modification by Borchgrevink and Waaler (10) is a much more sensitive method It is prolonged also in mild forms of the disease where the Duke bleeding time is normal (54 61)

Measurement of platelet adhesiveness is now widely used to diagnose von Willebrand s disease Salzman s method is used most and its value is well documented (1 42 57 60 64) As a rule patients with von Willebrand s disease have low values below 20% but there is a certain overlap between normal persons and those with von Willebrand s disease We therefore feel that reduced platelet adhesiveness alone cannot be taken as evidence of a symptom free person having von Willebrand s disease or being a carrier of the disease even if typical disease is known in the family Determination of ristocetin co-factor activity with a quantitative method is often useful in the diagnosis provided the platelet function as such is normal Ristocetin co-factor is usually measured in a washed platelet system (70) We now use a system with formalin-fixed platelets and the plasma samples are diluted in von Willebrand plasma lacking ristocetin co-factor This method has worked well and has high reproducibility (77)

A firm diagnosis of von Willebrand s disease can not be made before one has studied the effect of an infusion of AHF concentrate (fraction I-0 or cryoprecipitate) at least in the proband in each family The Duke bleeding time should become shorter while the Ivy bleeding time is less responsive The retrograde increase in factor VIII activity is diagnostic but may sometimes be difficult to demonstrate in patients with mild von Willebrand's disease

Variants of von Willebrand's disease

It has been felt for some time that the concept of von Willebrand s disease might comprise a group of related disorders Zimmerman et al (75) in 1971 found that the disease was uniform in so far as the factor VIII antigen content in all their patients was low In 1972 however we demonstrated that a group of patients with the diagnosis of von Willebrand s disease was heterogenous since some of

Table IV Variant of von Willebrand's disease group 3

	Factor VIII activity (%)	Factor VIII antigen (%)	Bleeding time (Ivy) (min)	Platelet retention (Sz) (%)	Ristocetin co-factor (%)
Patient	88	12	>30	13	45
Mother	120	49	12	19	50
Father	115	47	14	4	92
Normal	60–160	50–175	6–12	20–60	>50

them had a normal level of factor VIII antigen (28) The existence of genetic variants of von Willebrand s disease has then been recognized by several authors

The variant patterns or subtypes of von Willebrand s disease have been described by the parameters connected with the factor VIII molecule complex (Table III) We have now re-examined more than 150 of the Swedish patients with a diagnosis of von Willebrand s disease (31) Factor VIII antigen measured with the rocket method is low in the majority of patients We could assign 40 families with altogether 101 affected members to a type of von Willebrand s disease which is characterized by a low factor VIII antigen and a proportionate decrease in factor VIII activity The bleeding time is always prolonged and platelet retention in a glass bead column is decreased or absent There is also a proportionate reduction of ristocetin co-factor The response to infusion of factor VIII concentrate is the one considered typical of von Willebrand s disease with a retarded increase or a persisting level of factor VIII activity (Fig 5) Six patients among the 101 in this group had very severe symptoms In these six patients factor VIII antigen could not be detected in the plasma with Laurell s technique No antigen could be detected in the platelets or in the vascular intima We have found an abnormal electrophoretic mobility of factor VIII antigen in a few of the patients belonging to this group

Among the descendants of von Willebrand s original family there is no living one with a severe form of the disease as far as we know We have examined a few of the descendants now living in Stockholm One affected had a low level of factor VIII activity as well as antigen and ristocetin co-factor The families which we have grouped together as type 1 therefore seem to have the genuine von Willebrand s disease The inheritance in this group is no doubt autosomal and dominant This classical von Willebrand s disease is the largest group

Several workers have reported on von Willebrand patients who regularly have normal levels of factor VIII antigen as determined by the rocket method of Laurell (Table III) We have 4 families in which the rather severely affected members usually have a level of factor VIII antigen above the lower border of 50% Factor VIII activity is either slightly low borderline or low normal Bleeding time is prolonged platelet retention reduced and ristocetin aggregation low or subnormal The response to infusion of factor VIII is typical of von Willebrand s disease Factor VIII antigen is present in the platelets Although some of the patients assigned to this group were severely affected they had the antigen in their vascular endothelium The protein has an abnomal electrophoretic mobility as demonstrated by crossed immunoelectrophoresis It would appear that this abnormal protein exists in a more loosely aggregated form than the normal protein In only one of these four families could the inheritance be decided but then it was autosomal and dominant These families resemble those described by Koutts et al (41) Peake et al (55) Kernoff et al (40) and Thomson et al (66) Peake et al and Kernoff et al were the first to find that this variant of von Willebrand s disease had a protein with abnormal electrophoretic mobility Gralnick et al (22) have recently described two similar families We have assigned this variant to group 2

Now when we have examined a large amount of patients a third type has become obvious Six patients from five families have a pattern which is shown as group 3 (Table III) Factor VIII antigen is reduced out of proportion to factor VIII activity which is quite normal The pattern has been especially evident in two severely [illegible] one of them a girl who had life [illegible]

at menarche is presented in Table IV She filled the criteria for von Willebrand s disease except that she had normal factor VIII activity on repeated occa sions The antigen was very low It was remarkable that the ristocetin aggregation was only slightly abnormal The patient s factor VIII activity eluted later on a column of Sepharose 2B than did the factor VIII activity from normal plasma This indi cates that her factor VIII activity is bound to an abnormally small molecule This type of disease may be autosomal and recessive The patient s par ents were first cousins and both had borderline factor VIII antigen and low platelet retention but were asymptomatic

Besides these variants we have found 7 families in which the affected members of both sexes had low factor VIII activity and prolonged bleeding time but normal factor VIII antigen and normal platelet aggregation with ristocetin (Table III group 4) The electrophoretic mobility of factor VIII antigen is normal in this group (27 31) We have found no platelet abnormality either as aggregation with collagen adrenaline and ADP was normal In at least 3 of these families the pedigrees strongly sug gest X chromosomal inheritance We now feel that the disease in these families should be classified as a variant of haemophilia A In one family ordinary haemophilia A coexisted with this variant type of bleeding disease (28) Patients who seem clinically *identical with this group have been described by* Koutts et al (41) but no details are available

Another variant has been reported by others (8 66 67) (Table III group 5) In these patients the factor VIII molecule complex appeared to be lack ing in both factor VIII activity and normal antigenic determinants but to possess von Willebrand factor activity (normal bleeding time) In our material we have not seen any such patients and we wonder whether one can call a disease with normal bleeding time von Willebrand s disease

Much work has to be done to clarify these var iants of the disease Then one can ask which criteria should really be adopted for a diagnosis of von Willebrand s disease In a quantitative defect of factor VIII protein complex all parameters are of course decreased and the diagnosis is often easy In supposed qualitative defects the results may not be so clear cut It is obvious that a diagnosis of von *Willebrand s disease cannot be made if only one* parameter is present and it is questionable when there are only two

Treatment

The discovery that von Willebrand s disease is due to lack of a plasma factor opened up a new approach to treatment namely administration of fresh plasma Good therapeutic and prophylactic results have been achieved but in severe von Wil lebrand s disease it has often proved to be less suitable because administration of sufficient vol umes to secure haemostasis can cause a strain on the circulation

The first acceptable concentrate was the Swedish fraction I–0 (51) This preparation has been used with success for almost 20 years and has proved capable of controlling all types of bleeding in von Willebrand s disease Cryoprecipitate has also been *reported to have a good effect*

The common inheritable haemorrhagic disorder, von Willebrand s disease, has been known for 50 years During these years it has been well es tablished that the cause of the disease is a defi ciency of a plasma protein with remarkable biologi cal properties We now know that the factor VIII related protein is composed of a subunit with a molecular weight of about 200 000 and about 1600 amino acid residues 10 or 20 or even more of these subunits make up the von Willebrand protein There are of course many possibilities of amino acid substitution in the large chain Different substi tutions may lead to a reduced tendency to form the large aggregates One can easily imagine the large variability in clinical symptoms and laboratory find ings in such a system If we are to make any advance in this sector we must isolate the subunits from normal and from von Willebrand plasma and compare their primary structures

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ADP-induced Platelet Aggregation In Vitro in Patients with Ischemic Heart Disease and Peripheral Thromboatherosclerosis

Johs Gormsen Jørn Dalsgaard Nielsen and Leif Agerskov Andersen

From the Coagulation Laboratory Sundby Hospital Copenhagen Denmark

ABSTRACT ADP induced platelet aggregation in vitro has been studied in 90 normal controls and in 30 patients with ischemic heart disease (IHD) and 22 with peripheral thromboatherosclerosis (PTA) The sensitivity to ADP was defined by the threshold concentration which produced secondary aggregation with an amplitude corresponding to not less than 80% of the transmission obtained by platelet poor plasma In the normal controls the threshold concentration was significantly lower in women aged 50 or more than in women under that age The geometric means were lower in the patients than in the controls Significantly lower threshold concentrations than in the corresponding age groups of controls were found in the following age groups of patients Men and women ≥50 years with IHD ($p<0.005$ and $p<0.001$, respectively) men and women under 50 with IHD ($p<0.05$) Men and women ≥50 years with PTA ($p<0.002$ and $p<0.01$ respectively) men and women under 50 with PTA ($p<0.005$)

The role of human platelets in the development and progression of arteriosclerotic lesions has been studied intensively in recent years Several authors (10 18) have shown that platelet turnover is selectively increased in patients suffering from ischemic heart disease (IHD) transient cerebral ischemia (TCI) or peripheral thromboatherosclerosis (PTA) Platelet aggregates have been demonstrated in circulating blood from patients with these disorders (25) as well as in the microcirculation of the lungs in acute death (21) and especially in the intramyocardial microcirculation in IHD patients who died suddenly (8)

One important question is whether the biological activity of the platelets is increased in these disorders Several authors using [illegible] have demonstrated an increa[illegible] tion in vitro after addition of [illegible] (ADP) epinephrine and co[illegible] ports on hyperactive plat[illegible]

The aim of the present [illegible] statistically the thresh[illegible] —the lowest concentrat[illegible] aggregation in vitro—[illegible] patients suffering from IH[illegible]

PLATELET AGG[illegible]

Platelet aggregat on in vit[illegible] according to the principles [illegible] The experiments were perf[illegible] aggregometer model 300 [illegible] ture after m n mal stasis [illegible] meals for 12 hours no [illegible] t on period of 30 m n) [illegible] w th one part of 3 1[illegible] Platelet rich and platele[illegible] mmediate centrifugat[illegible] at 200×g and for 10 [illegible] plasma was removed [illegible] room temperature in [illegible] tion studies were perf[illegible] at a st rring speed of [illegible] ing the plasma be ng [illegible] add t on of ADP T[illegible] ca 150 000–350 000 pl[illegible] counter chamber V[illegible] th s range d d not [illegible] was induced by ad[illegible] ml sterile phys olog[illegible] tions of ADP (A[illegible] USA) giving final [illegible] 2 0 and 5 0 μM Th[illegible] tion of ADP—the [illegible] duce secondary ag[illegible]

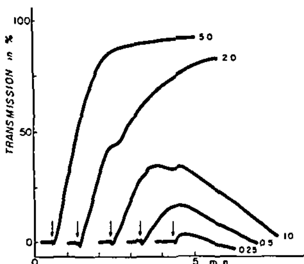


Fig 1 Turbidometric determination of platelet aggregation after addition of ADP in vitro With increasing ADP concentrations the threshold value is defined as the lowest concentration which produces a secondary aggregation with an amplitude giving a transmission not less than 80% of that corresponding to the platelet poor plasma (100%)

transmission not less than 80% of the transmission given by the platelet poor plasma

The results were identical when carried out in duplicate after repuncture and from day to day when performed at the same time of day All individuals tested were carefully asked for any intake of drugs which might influence platelet aggregation and were excluded if they had taken such drugs during the past week

Statistical analysis

The geometric means are presented for the ADP data The significance of the results in controls and in the two patient groups was evaluated by the non parametrical Mann Whitney *U* test for two independent samples

STUDY POPULATION

The *normal controls* were 90 persons 20–69 years old all donors at the Blood Bank Their sex and age distributions are given in Table I

Patients All patients were tested at least twice and all values given represent two identical results If duplicate experiments gave different results a third analysis was performed and the concentration obtained twice was chosen

Ischemic heart disease The group comprises 30 patients 16 women and 14 men (Table I) admitted to the Coagulation Laboratory due to progression of the clinical symptoms The diagnosis of IHD was based on a typical history with one to two attacks of angina pectoris a day responsive to nitroglycerin and *absence* of valvular heart disease hypertension congestive heart failure chronic obstructive pulmonary disease diabetes mellitus anemia polycythemia elevated serum creatinine and biochemically determined hepatic disease If the diagnosis was not clinically obvious definite ECG evidence of myocardial ischemia associated with ST depression and chest pain during submaximal exercise test was demanded Five of the patients had suffered from acute myocardial infarction (AMI) more than 6 months previously Three patients had hyperlipoproteinemia type II and one type IV

Peripheral thromboatherosclerosis The group includes 22 patients admitted to the Coagulation Laboratory with typical claudicatio intermittens (Table I) In 12 patients arteriography had shown a thrombus formation and atherosclerosis in the iliac and femoral arteries in the remaining 10 the diagnosis was verified by measuring a reduction of the arterial BP of the toe to less than 40 mmHg (the Department of Clinical Physiology Bispebjerg Hospital) Patients with diseases which did not fulfil the above criteria were excluded

RESULTS

Normal controls The distribution of the threshold concentrations of ADP is given in Table I and Fig 2 Among men the threshold does not differ significantly between those under and over 50 years of age respectively ($p>0.1$) Among women those over 50 have a significantly lower threshold than those under 50 ($0.01<p<0.025$) Men and women under 50 do not differ significantly in their threshold concentration ($p>0.05$) neither do men and women over 50 ($p>0.1$) The geometric mean is 2 14 for men under 50 2 92 for women under 50 1 70 for men over 50 and 1 31 for women over 50

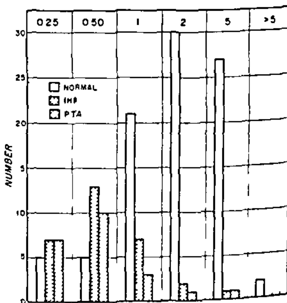


Fig 2 Threshold concentration of ADP (μM) in normal controls and in patients

Table I *Threshold concentrations of ADP in controls patients with IHD and patients with PTA*

Age (y)	ADP concentration (μM) 0 25 ♀	0 25 ♂	0 5 ♀	0 5 ♂	1 0 ♀	1 0 ♂	2 0 ♀	2 0 ♂	5 0 ♀	5 0 ♂	>5 ♀	>5 ♂	Total
Controls													
20–29						1	1	1	2	2			7
30–39		1	2			3		3	5				14
40–49						1	1	8	1	6			17
50–59	1			3	5	4	1	12	1	7		2	36
60–69		3			2	5	1	2	1	2			16
≥70													
	1	4	2	3	7	14	4	26	10	17	0	2	
Total	5		5		21		30		27		2		90
Patients with IHD													
20–29													
30–39													
40–49			1			3			1				5
50–59	2		3	3	1	1							10
60–69	3	1	3	2		2		2					13
≥70	1		1										2
	6	1	8	5	1	6	0	2	1	0			
Total	7		13		7		2		1				30
Patients with PTA													
20–29				1									1
30–39				1									1
40–49	2				1								3
50–59	1		1	2	1	1		1		1			8
60–69	1	3	1	3									8
≥70			1										1
	4	3	3	7	2	1	0	1	0	1			
Total	7		10		3		1		1				22

Ischemic heart disease Threshold concentrations are given in Table I and Fig 2 Among men over 50 those with IHD have a significantly lower threshold concentration than the normal controls ($p<0\ 005$) The same applies to women over 50 ($p<0\ 001$) The threshold for the women over 50 with IHD is significantly lower than that for the men over 50 with IHD ($0\ 01<p<0\ 025$) Patients under 50 with IHD (men+women) have a significantly lower threshold than the controls under 50 ($p<0\ 05$) In general the lowest threshold concentrations were found in patients with more progressive symptoms The threshold concentration was 0 25 μM in the three patients with hyperlipoproteinemia type II Exclusion of them does not significantly influence the statistical findings The geometric means are men under 50 years 1 00 women under 50 years 1 58 men over 50 years 0 73 and women over 50 years 0 39

Peripheral thromboatherosclerosis The ADP threshold concentrations are given in Table I and Fig 2 Among men over 50 those with PTA have a significantly lower threshold dose than the normal controls ($p<0\ 002$) The same applies to women over 50 ($0\ 001<p<0\ 01$) There is no significant difference between men and women over 50 with PTA ($p>0\ 05$) The threshold concentration in PTA patients (men+women) under 50 is significantly lower than in the corresponding age groups of controls ($p<0\ 005$) The geometric means are men under 50 years 0 50 women under 50 years 0 40 men over 50 years 0 62 and women over 50 years 0 45

DISCUSSION

The present study has shown that it is possible by standardizing the experimental conditions carefully to perform reproducible aggregation experiments in terms of the threshold concentration of ADP which produces a secondary platelet aggrega

tion followed by a maximal amplitude corresponding to a light transmission of not less than 80% of that given by the platelet poor plasma

The distribution of the threshold values in the normal controls is biologically reasonable with a geometric mean of around 2 μM In general threshold concentrations were lower in elderly people and especially in elderly women This difference was significant for the women over 50 Hardistry et al (9) found a lower normal range (0 2–1 4 μM) However they selected the threshold dose which gave a distinct second curve but with a very low secondary amplitude Frishman et al (6) report a geometric threshold concentration of 3 85 μM (11 normal controls aged 41–60) they selected concentrations which gave maximal amplitude

The present study has furthermore verified that platelets in platelet rich citrated plasma in vitro aggregate at a lower concentration of ADP if the blood is collected from patients suffering from IHD or PTA than if it is drained from normal controls Again the lowest values were found in women over 50 We have previously demonstrated that lower ADP threshold concentrations also are found in patients suffering from TCI (1) and in patients recovering from acute cerebral infarction (15) Frishman et al (6) found a similar lower threshold concentration in patients with angina pectoris Several other authors using various modifications of platelet aggregation methods have demonstrated an increased aggregation tendency in patients with AMI with IHD without AMI with cerebral infarctions TCI or PTA (3 5 7 16 23 26 27) Increased platelet aggregation has also been described in patients with diabetes mellitus and especially if this is complicated by retinopathy nephropathy or neuropathy possibly due to a factor in the plasma (13 20 22) The increased aggregation tendency in patients with hyperlipoproteinemia type II (4) might be due to incorporation of cholesterol into the platelet membranes (24) This increase can be changed by altering the lipid pattern of the plasma (12 19) Smoking (14) and elevation of the catecholamines and the free fatty acids in plasma increase the aggregation tendency temporarily (17)

It is difficult to evaluate the possible significance of this hyperaggregability in vitro The increase in the aggregation tendency is especially seen in patients with a high frequency of arterial thrombi and particularly in periods with progression of the clinical symptoms Also it is not known whether the changes are primary and/or secondary to the basic disease The question whether the increase in aggregation tendency is an indication for treatment with drugs that inhibit platelet activity, remains to be answered (11)

ACKNOWLEDGEMENT

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The Peripheral Platelet Count in Response to Intravenous Infusion of Salbutamol

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ABSTRACT **Five healthy male volunteers received i v infusions of salbutamol a relatively selective β 2 receptor stimulating agent, in doses of 0 03, 0 06, 0 09 and 0 27 $\mu g \times kg^{-1} \times min^{-1}$ over a period of 6 min At the three low doses the heart rate remained essentially unchanged and no significant decrease in the platelet count occurred However in response to 0 27 $\mu g \times kg^{-1} \times min^{-1}$ of salbutamol the heart rate increased by 25% over basal value ($p<0$ 01) and a significant lowering ($p<0$ 005) of the platelet count was obtained The present findings suggest that the drop in the peripheral platelet concentration in response to adrenergic β receptor stimulation is mediated via β 1 receptors**

Recently we reported that an i v infusion of isoprenaline a non selective adrenergic β receptor stimulator caused a significant decrease in the peripheral platelet concentration (2) It was also suggested that this effect on the platelets was a result of an adrenergic β receptor mediated increase in the size of the exchangeable splenic platelet pool (ESPP) Isoprenaline is known to stimulate β 1 and β 2 receptors to about the same extent

The aim of the present study was to clarify whether β 1 or β 2 receptors are involved in the above phenomenon Therefore a selective β 2 receptor stimulating agent salbutamol was given i v in increasing doses and its effect on the peripheral platelet count was investigated

STUDY POPULATION AND METHODS

The study comprises 5 healthy male volunteers aged 22–33 years (mean 27) They all belonged to the medical staff of our department and their fully informed consent had been sought and obtained

After a light breakfast the subjects arrived at the laboratory at 8 a m Catheters were inserted into the antecubital veins and the subjects rested in supine position for 10 min At intervals of about 2 weeks 5 studies were carried out in each subject In the experiments 4 different doses of salbutamol (Glaxo Molndal Sweden) or 30 ml of saline were randomly infused During the experiment venous blood was collected at 5 min intervals for platelet count and determination of haematocrit ECG was continuously monitored and BP was recorded every 5 min Before infusion two baseline samples were taken for platelet count and haematocrit value Thereafter either 30 ml of saline or l salbutamol diluted in 30 ml saline were infused i v in doses of 0 03 0 06 0 09 and 0 27 $\mu g \times kg^{-1} \times min^{-1}$ over a period of 6 min For this purpose a constant flow pump device was employed At each experiment 9 blood samples were drawn The enumeration of platelets was carried out by phase microscopy (1) and the haematocrit values were obtained by centrifugation of blood in capillary tubes at 13460 *g* for 5 min (IEC MB Micro-Hematocrit Centrifuge)

Standard statistical methods were used Unless otherwise stated mean values ± standard error of the mean (S E) are reported Statistical analyses were made using Student s *t* test and calculating differences between matched pairs The difference between means was considered significant if $p<0$ 05

RESULTS

Heart rate Fig 1 shows the change in heart rate in response to increasing doses of salbutamol recorded during the 6th min of infusion The lowest two doses did not cause any significant change At the two highest doses heart rate increased by 9 and 25% respectively over basal value These two differences were statistically significant ($p<0$ 02 and $p<0$ 01) For comparison the corresponding results

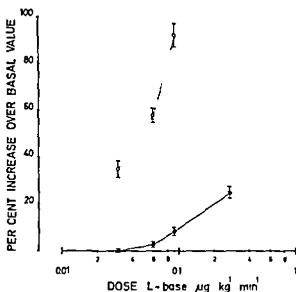


Fig 1 Change in heart rate during i v infusions of 4 different doses of salbutamol (●—●) and 3 different doses of isoprenaline (O O) to 5 healthy male subjects (mean ±S E) The latter results from Olsson et al (2)

obtained with isoprenaline in 5 healthy volunteers (2) four of whom participated in the present investigation are also given in Fig 1 It should be noted that even at the lowest dose of isoprenaline (0 03 $\mu g \times kg^{-1} \times min^{-1}$) a 35% increase in heart rate was recorded

Peripheral platelet count The results obtained with the saline and the four salbutamol infusions are shown in Fig 2 The platelet count in the first of the two baseline samples was arbitrarily set as 1 0 and all subsequent values were related to this In response to saline and the two lowest doses of salbutamol no change in the peripheral platelet count occurred A tendency to decreasing platelet values was present when salbutamol was infused in a dose of 0 09 $\mu g \times kg^{-1} \times min^{-1}$ but the lowering was not statistically significant However in response to salbutamol in a dose of 0 27 $\mu g \times kg^{-1} \times min^{-1}$ a significant decrease ($p<0\ 005$) in the peripheral platelet count was observed and a lowest value was obtained 10 min after the start of the infusion The results of these infusion tests are summarized in Table 1 together with the corresponding results from our recent isoprenaline study (2) During the experiments there was no change in the venous haematocrit

Table 1 *Platelet count in response to different doses of i v salbutamol and isoprenaline infusions given to 5 healthy male volunteers (mean ± S E)*

Dose ($\mu g \times kg^{-1} \times min^{-1}$)	Peripheral platelet count ($\times 10^9$/l) Baseline value	Lowest value	*P*
Salbutamol			
0 03	184±17	174±15	n s
0 06	208±29	195±22	n s
0 09	192±26	173±18	n s
0 27	200±26	172±23	<0 005
*Isoprenaline*ᵃ			
0 03	197±18	178±17	<0 02
0 06	211±19	177±20	<0 02
0 09	203±24	183±24	<0 02

ᵃ From Olsson et al (2)
n s = not significant

DISCUSSION

Isoprenaline and salbutamol are known to be about equipotent in vivo with respect to the β 2 receptor

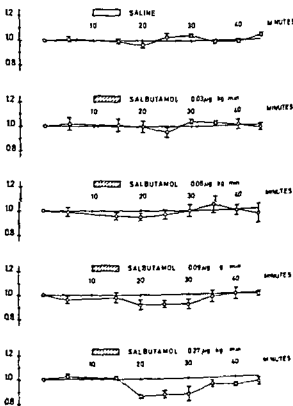


Fig 2 Peripheral platelet count in response to saline and salbutamol infusions given to 5 healthy male volunteers (mean ±S E)

mediated effect on the human bronchial smooth muscles (expressed as increase in $FEV_{1.0}$) but differ considerably with respect to the β 1 mediated effect on the heart as estimated by Svedmyr and Thiringer (3 4) who also estimated ED 50 for the effect on the bronchial smooth muscles to about 0 01–0 02 μg×kg ⁻¹×min ⁻¹ The increase in heart rate observed when these ED 50 doses were administered was about 35% for isoprenaline and not significant for salbutamol When larger doses were infused about 10 times as much salbutamol had to be given to increase the pulse rate percentage wise as much as the $FEV_{1.0}$ thus indicating a relative selectivity for the β 2 receptors for salbutamol

We infused isoprenaline in doses of 0 03 0 06 and 0 09 μg×kg ⁻¹×min ⁻¹ over a 6-min period to 5 healthy young males and observed a statistically significant drop in the peripheral platelet count (2) The maximal average lowering in platelet count varied between 10 and 16% and the maximal response appeared to have been reached already at the lowest dose used (Table I) At this dose (0 03 μg×kg ⁻¹×min ⁻¹) the increase in heart rate was about 35% (Fig 1) When salbutamol was administered in the same doses and over the same period as isoprenaline the platelet count was not significantly affected Similarly the heart rate was essentially unchanged at these low doses However in response to salbutamol in a dose of 0 27 μg×kg ⁻¹×min ⁻¹ a significant decrease in the platelet count was obtained the reduction amounting to 14% of the base line value Simultaneously an increase in heart rate of about 25% was observed Hence at increasing doses of salbutamol a stimulation of the β 1 receptors in the heart was also achieved The same dose level elicited an effect on the peripheral platelet count These findings suggest that the increase in heart rate and the lowering of peripheral platelet concentration are mediated via the same type of receptors The mechanism behind this adrenergic β 1 receptor mediated effect on the ESPP is currently under investigation in our laboratories

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Raynaud's Phenomenon

Photoelectric Plethysmography of the Fingers of Persons with and without Raynaud's Phenomenon during Cooling and Warming up

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ABSTRACT A study has been made of the changes in the amplitudes shown on photoelectric plethysmographs of the fingers of one hand of controls (17 men and 17 women without Raynaud's phenomenon) during cooling and warming up of the hand This is called the cooling and warming up test The hands of the women appeared to cool more quickly than of the men, besides getting warm more quickly and remaining warm for a longer period This difference is already apparent at the lowest water temperatures (6 and 3 C) and may be ascribed to a difference in hand volume, with a hunting reaction due to extreme cold The lowest values of controls appear to be useful for the clinical differentiation of patients with a serious Raynaud's phenomenon The test is said to be positive when the values are below the lowest values of the controls The test was performed on 50 of 58 out patients suffering from Raynaud s phenomenon In serious cases the severity of the disease could be assessed objectively In less serious cases the test was sometimes negative

In patients with Raynaud s phenomenon discolorations of the fingers and toes occur when these parts of the body turn cold and subsequently become warm in successive phases of white blue and red Sometimes the red phase is preceded by either a blue or a white phase only It is extremely difficult to determine the seriousness of the complaint Moreover the pain expressed by the patients varies widely (1)

In our clinic for several years now the diagnosis of Raynaud s phenomenon has been made by means of photoelectric plethysmography of the fingers during cooling of the hand in ice cold water Disappearance of the amplitude in the plethysmogram after immersion of the hand in ice-cold water for 5 min is taken as evidence that Raynaud s phenomenon is present (2 3 4 5) The aim of our study was to achieve a greater differentiation in the changes of the amplitudes by cooling and warming up the hand more slowly so that the severity of the disease could be determined more precisely If so this would provide us with a diagnostic tool and might enable us to assess the effects of treatment objectively (7)

The group of patients suffering from Raynaud s phenomenon includes patients with LE scleroderma and Raynaud s syndrome The latter is defined by Allen et al as Raynaud s phenomenon without associated and contributing conditions and diseases (1)

STUDY POPULATION

Controls of the test were 17 men and 17 women in good health and thus without Raynaud s phenomenon The mean age was 43 years (range 20–72) for men and 41 years (range 19–70) for women (Table I)

As to the 58 out patients who were referred to our department because of cold and pallor or blue fingers the mean age of the 26 men was 49 years (range 24–66) and of the 32 women 46 years (range 17–78) Five had LE (3 men 2 women) 15 scleroderma (5 men 10 women) and 38 Raynaud s syndrome (18 men 20 women) (Table II)

All patients were controlled by photoelectric plethysmograms after warming up The patients suffering from pure Raynaud s syndrome and those with LE showed a normal photoelectric plethysmogram Of the 15 patients with scleroderma 10 suffered from a severe scleroderma and showed an abnormal photoelectric plethysmogram In 8 patients the cooling test was omitted as being too painful their photoelectric plethysmogram showed serious abnormalities at a water temperature of 33 C

Table I *Age and hand volume of the controls*

Case no	Men: Age (y)	Men: Hand volume (ml)	Women: Age (y)	Women: Hand volume (ml)
1	59	250	60	200
2	37	250	55	200
3	69	225	70	175
4	49	225	57	150
5	48	225	53	150
6	60	200	53	150
7	56	200	46	150
8	38	200	45	150
9	45	175	40	150
10	41	175	20	150
11	34	175	53	125
12	32	175	23	125
13	22	175	23	125
14	20	175	23	125
15	72	150	20	125
16	25	150	19	125
17	20	150	39	100
Mean	43	193	41	146

METHODS

The test was carried out on all fingers of one hand. The subjects lay recumbent for 20 min before any observations were made. During this time the recorder apparatus is applied. The time needed for proper measurements is about one hour. The room temperature is 25 C. A light dependent resistance (LDR) element (Lode, Groningen, The Netherlands) is applied to each finger (Fig. 1). Then the hand is immersed in the water reservoir of a specially constructed cooling apparatus (Laboratory of Medical Physics, University of Groningen). Every 4 min the reservoir is drained automatically and refilled with 3 C colder water, thus exposing the hand successively to 33, 30, 27 ... 3 C. All the time registrations are made with a paper speed of 0.25 mm/sec. At the end of each 4 minute period with water of a certain temperature, however, the paper speed is accelerated for about 4 sec to 25 mm/sec, to get a more detailed picture of the curves. Disappearance of the amplitude at a certain temperature is easily determined (Fig. 2).

After immersion of the hand in water of 3°C for 4 min the water is drained again and the wet hand is exposed to the environmental air temperature in the water reservoir which is covered with a piece of cloth. During the following 10 min the amplitude of the plethysmogram is measured each minute, in order to register the time course of the relaxation of the contracted arterioles. Exposure of the wet hand to air is not ideal, but in this position there seems to be no other way of obtaining an acceptable standardization of the procedure without interfering excessively with the posture and position of the hand and the LDR elements.

The photoelectric plethysmogram does not measure the skin bloodflow of the fingers quantitatively. It simply indicates the changes in volume of skin vessels. A rapid increase and decrease in the amplitude of the plethysmogram correlates with an increase and a decrease in the bloodflow of the fingers (3). However, the amplitude depends to some degree on the filling of the venous vessels and consequently on the pressure of the LDR element on the skin and on the posture of the hand in relation to the heart (2, 5, 6). In order to eliminate these factors, the initial amplitude of the photoelectric plethysmogram, when the hand is immersed in water of 33°C, is set at 100 and the successive values are represented as percentages of

Table II *Age and sex distribution of the patients*

Figures in italics indicate patients without cooling and warming up test

Age (y): Men	Women	Men	Women
Lupus erythematosus (n=5)		*Raynaud's syndrome (n=38)*	
48	48	24	17
62	60	41	21
66		41	24
		43	24
Scleroderma (n 7+8)		44	27
		45	27
40	65	45	31
41	78	46	32
43	*20*	48	35
46	*30*	49	38
63	*60*	52	39
	62	52	43
	64	52	44
	64	54	46
	65	55	51
	70	58	53
		60	54
		62	54
			60
			61
Mean 51	62	48	39



Fig. 1. LDR elements.

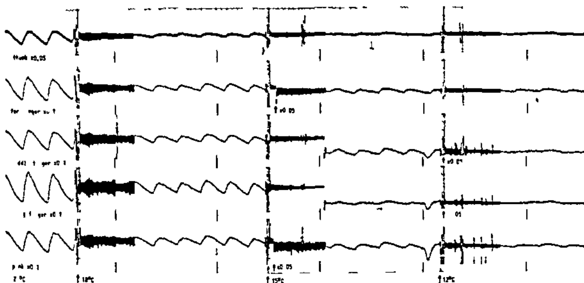


Fig 2 Photoelectric plethysmograms of the 5 fingers of a patient during the cooling period Paper speed 0 25 and 25 mm/sec after each 4 min room temperature 25°C ×0 1 and ×0 05 are the sensitivities of the recorder The amplitude has disappeared at a temperature of 12°C in the thumb middle finger and ring finger

this initial value The curves for an individual are reproducible as was shown in some preliminary experiments

RESULTS

Controls

The sex age and hand volume of the controls are presented in Table I Medians and lowest values of the percentage amplitudes per cooling step and warming up per minute are given for men and women separately (Fig 3) All amplitudes of the men appear to differ significantly from those of the women at water temperatures of 27 24 21 18 15 and 3 C and also during the warming up period ($p<0.01$) and at 6 C ($p<0.05$) (Mann Whitney U test)

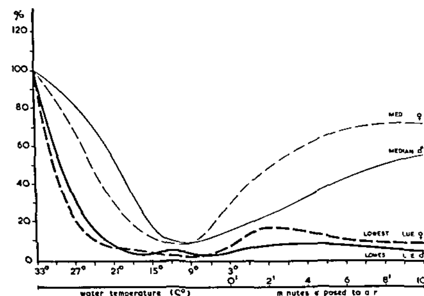


Fig 3 Median and lowest values of the amplitude of the photoelectric plethysmogram for men and women without Raynaud s phenomenon

Table III *Scoring of percentages of the amplitudes of the cooling and warming up plethysmograms*

Above = negative below = positive
The lowest values of the controls can be defined as the lowest amplitudes in percentages as expressed on the plethysmogram of a finger

Score	Cooling	Warming up
0	Remains above the lowest values	Remains above the lowest values
1	About equal to the lowest values	About equal to the lowest values
2	2–3 fingers drop out	2–3 fingers remain temporarily below the lowest values
3	4–5 fingers drop out	2–3 fingers remain permanently below the lowest values
4	2–3 fingers drop out before 18°C	2–3 fingers do not recover in 10 min
5	4–5 fingers drop out before 18°C	4–5 fingers do not recover in 10 min

The lowest values of the controls are taken to be the lowest normal values the differences between men and women being taken into account

Patients

Pathological changes in the cooling and warming up test were scored according to Table III Scores of 0 and 1 during the cooling period and of 0 1 and 2 during the warming up were considered to be normal Other scores are abnormal

Fig 4 shows that of the 50 patients with Raynaud s phenomenon 21 do not have any deviant curves and 29 do In the 38 patients without LE sclero-derma or other disorders i e patients with Ray naud s syndrome the ratio is 19 19 in the 5 LE pa tients 1 4 and in the 7 scleroderma patients 1 6 When the 8 patients who were most seriously ill are included the ratio is 1 14

DISCUSSION

Among the controls remarkable differences were found between the curves of the men and the women The increase in amplitude which has already started during the cooling period at 6–3°C is greater in women than in men This might be due to a sort of hunting reaction The differences might be attributable to the difference in hand volume between men and women (Table I) The hand volume of men is an average of 47 ml more than that of women Due to their smaller hand volume the cooling is more intense in women consequently the hunting reaction may set in earlier

In each group of patients (Raynaud s syndrome LE scleroderma) there were individuals with Raynaud s phenomenon whose cooling and warming up test was negative so in these cases the test was not sensitive enough The test is carried out at a room temperature of 25°C Cooling of the whole body might give better results However in the cases in which objectivation was possible the method enabled us to demonstrate gradients of severity of Raynaud s phenomenon (Fig 4) Furthermore it was evident that the scores obtained by these tests correlated with the severity of the symptoms presented by the patients since the tests are more positive in patients with LE and scleroderma In patients with Raynaud s syndrome there are no clinical symptoms which predict the results of the test Fig 4 also shows that serious divergences during the warming up period (scores 3–5) hardly ever occurred in patients with an evident drop-out of all fingers of one hand (cooling 3–5) there is only one exception (cooling 1 warming up 3) On the other hand there are 21 patients with drop-out of all fingers at lower temperatures (cooling 3) 10 of whom quickly regain normal curves during the warming up period (warming up

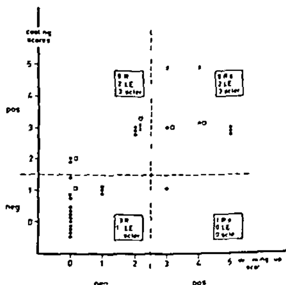


Fig 4 Scores of the 50 patients with Raynaud's phenomenon ● = Raynaud's syndrome □ = LE × = scleroderma

0 1 and 2) and 11 keep small amplitudes (warming up 3 4 and 5) for an abnormally long period The reason why these patients react so differently is not yet clear

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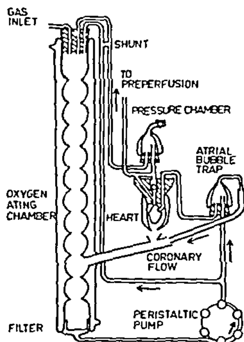


Fig 1 Perfusion apparatus for isolated working rat heart under ischemic and non ischemic conditions Aorta and left atrium are cannulated The one way ball valve is placed just distal to the coronaries This ball valve can be bypassed A Windkessel effect is provided by a partly air filled chamber connected to the aortic tube The heart is pumping perfusate to the top of the oxygenating chamber A peristaltic pump provides the atrial bubble trap with perfusate The height of this bubble trap is adjustable and determines the atrial filling pressure Clamping of the bypass of the one way valve inhibits the diastolic perfusion of the coronaries thus inducing ischemia (Reproduced with permission from Recent Advances in Studies on Cardiac Structure and Metabolism vol 10 (ed P E Roy & G Rona) p 307 University Park Press Baltimore 1975

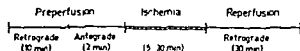


Fig 2 The time of preischemic perfusion was the same for all hearts (10+2 min) Time of ischemia varied from 5 to 30 min for different groups of hearts Retrograde perfusion after the ischemia was 30 min for all

the oxygenating cylinder (Fig 1) Hearts which were unable to produce an aortic pressure of 80 cm H_2O as a cause of ischemic damage were thus provided with a constant perfusion pressure The retrograde perfusion period was chosen to allow for accumulation of released enzymes in the buffer and for recovery of myocardial function The perfusions were performed according to the time schedule shown in Fig 2 The cardiac output and coronary flow

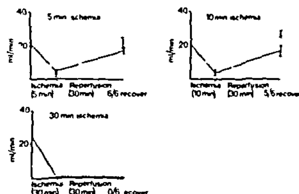


Fig 3 Effects of different times of ischemia on coronary (—) and aortic flows ( ) for 6 hearts paced at 300 beats/min (mean±S E)

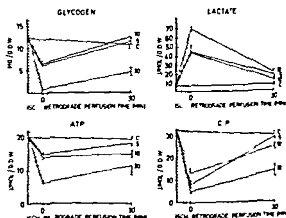


Fig 4 Tissue glycogen lactate ATP and creatine phosphate (*CrP*) after ischemia for 5 10 or 30 min (Ω) Controls were non ischemic All hearts (*n*=6–8) were paced at 300 beats/min during the time of ischemia and unpaced during the retrograde perfusion (mean±S E) (Reproduced with permission from Recent Advances in Studies on Cardiac Structure and Metabolism vol 10 (ed P E Roy & G Rona) p 307 University Park Press Baltimore 1975

were measured by collecting the perfusate in a graduated cylinder and calculating the volume per unit of time The buffer dropping from the heart was considered to be the coronary flow

Biochemical analysis

At the end of the experiment all hearts were frozen with a Wollenberger clamp cooled to the temperature of liquid nitrogen The heart specimen was then hammered to a fine powder in a percussion mortar previously cooled in liquid nitrogen A sample of this powder was taken for biochemical analysis Glycogen was determined according to a glucose oxidase method using rabbit liver glycogen as standard (36) Lactate was determined by the enzymatic method of Lundholm et al (29) ATP and creatine phosphate contents were measured by an enzymatic method described by Lamprecht and Trautschold (26)

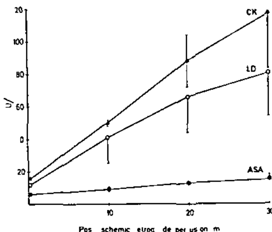


Fig 5 Release of ASAT LD and CK from 5–8 hearts after 5 min of ischemia and 30 min of reperfusion (mean+S E) * significantly different from initial value ($p<0.05$)

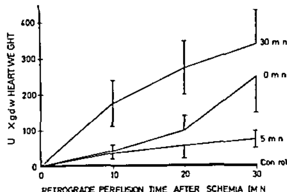


Fig 6 Release of ASAT from 6–8 hearts after ischemia for 5 10 or 30 min and subsequent perfusion for 30 min in a retrograde non working way (mean+S E) Controls were non ischemic throughout the whole perfusion

Buffer samples of 1 ml were taken 0 10 20 and 30 min after the start of the postischemic reperfusion period The samples were assayed on the same day for aspartate aminotransferase (ASAT EC 2 6 1 1) L lactate NAD oxidoreductase (LD EC 1 1 1 27) and creatine phosphokinase (CK EC 2 7 3 2) using an LKB 8600 Reaction Rate Analyzer (LKB Bromma Sweden) and standard UV test kits (Biochemical Test Combination Boehringer Mannheim GmbH West Germany) at 35 C

Distribution of flow

Two control and four ischemic hearts were perfused with buffer containing 10–15 μCi of ^{3}H antipyrine (New England Nuclear Corporation Boston USA) and Cardio Green (Hynson Westcott & Dunning Inc Baltimore USA) One ml of this solution was rapidly injected in the aortic tube just above the ball valve not until the ischemic hearts had reduced their coronary flows by about 60% As soon as the heart was visibly green it was momentarily frozen in isopentane precooled in liquid nitrogen The tissue was then sliced in a cryostat (20 to 25 C) in slices 10 or 15 μm thick Two different autoradiographic methods were then employed In method I (5 22) the sections were mounted on glass and heated to 65–70 C for about 30 sec They were then placed in close contact with dental X ray film (Kodak) and exposed at room temperature together with a drying agent (silica gel) for 2–4 weeks Control experiments to rule out any marked diffusion artefacts in this autoradiographic technique have been reported earlier (22) In method II the hearts were cut in a dark room The time slices were either adhered with a light touch to emulsion coated slides (Ilford K?) warmed to −5 C or thawed onto the slides by the finger tip (I 14) The autoradiographs were then exposed at 20°C for varying periods (4–6 weeks) the time of exposure depending on the amount of activity in the heart tissue Method II was used to achieve a resolving power of the autoradiographs on the cellular level After development the slices obtained by both methods were stained with Van Gieson and embedded in DePeX

Student s *t* test was used for statistical analysis A *p* value ≤0 05 was considered significant in this study

RESULTS

In one group the hearts were working for 10 min paced at 300 beats/min In a second group they were working unpaced for 30 min and in a third for 30 min while paced at 300 beats/min At the end of the perfusions there were no differences in myocardial content of creatine phosphate and ATP There

Table I *Effects of work with and without pacing on metabolites in six hearts (mean+S E)*

All hearts were perfused retrogradely for 30 min after the working period d w dry weight CK=creatine phosphokinase

Duration of work (min)	Pacing (beats/min)	Glycogen (mg/g d w)	Lactate (μmol/g d w)	ATP μmol/g d w)	Creatine phosphate (μmol/g d w)	CK (U/l)
10	300	13+1	8+1	?1+1	?3+3	14+6
30	Unpaced	11+1	16+1	19+1	1	17
30	300	6+1	13+3	0+1	1	1

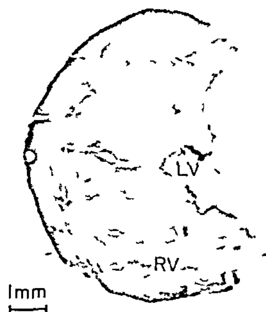


Fig 7 Distribution of ^{3}H antipyrine in a perfused control heart The distribution appears to be even at this magnification *LV* = left ventricle *RV* = right ventricle



Fig 9 Distribution of ^{3}H-antipyrine of an ischemic rat heart *The distribution appears even at this magnification* The subendocardial area is not reached by ^{3}H-antipyrine at the time of freezing *RV* = right ventricle *LV* = left ventricle

was a decrease in glycogen content for hearts working for 30 min while paced at 300 beats/min An increase was observed in lactate content for both groups working for 30 min There was a very small leakage of CK in all three groups (Table I) Almost immediately after the onset of ischemia there was a rapid fall in coronary as well as aortic flow The flow rates were restored for all hearts that were ischemic for 5 or 10 min but not for those that were ischemic for 30 min (Fig 3)

At the end of ischemia a decrease in myocardial glycogen content was found in all groups of hearts but it was restored after 30 min of reperfusion in all groups except that with 30 min of ischemia (Fig 4)

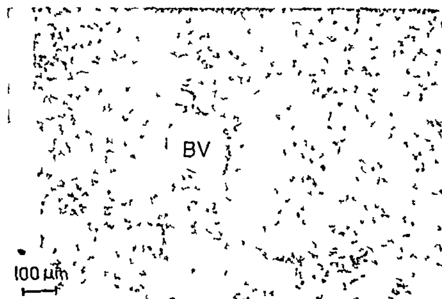


Fig 8 Distribution of ^{3}H antipyrine in perfused control hearts appears even at the cellular level too *BV* = blood vessel



Fig 10 Distribution of ^3H antipyrine of an ischemic rat heart at the cellular level The even distribution of flow between the cells is clearly visible as judged by this method

Myocardial lactate increased during ischemia but normalized again in all groups during the reperfusion period ATP and creatine phosphate levels decreased at the end of the ischemic period in all groups Only in the hearts with 5 min of ischemia were approximately normal levels attained during the reperfusion period The release of ASAT LD and CK to the perfusion buffer during 30 min of retrograde perfusion from hearts ischemic for 10 min is seen in Fig 5 A significant release of all three enzymes could be seen as early as after 10 min of reperfusion No difference was found in the time of onset of leakage of the three enzymes Fig 6 shows the leakage of ASAT after 5 10 and 30 min of ischemia In working controls there was a negligible leakage of enzymes Increasing time of ischemia was accompanied by an increasing leakage of enzymes

The flow of perfusate in the control situation was evenly distributed within the cardiac tissue as illustrated in Fig 7 at low magnification and Fig 8 at the cellular level Exactly the same uniform distribution is seen during ischemia in the parts of the tissue that were reached by the front of the isotope labelled perfusate (Figs 9 and 10) However due to the low velocity of the flow in the ischemic heart this front usually had not reached the whole endocardium at the time of freezing Thus an isotope free subendocardial zone of varying width was seen in these experiments (Fig 9)

DISCUSSION

It is not feasible to study the myocardial infarction process at the cellular level in man Since the mechanism for the development of myocardial infarction in man is not fully understood (2 8 9 10) it is difficult to choose a relevant experimental model In this study the isolated rat heart made ischemic by the use of an aortic ball valve (33) was used for the following reasons The experimental conditions can be standardized with respect to degree and length of ischemia Since the whole heart is made ischemic with the measured coronary flow representing the flow of the ischemic myocardium i e without any possibility of a compensatory flow to the normal myocardium the controls are fully comparable Metabolic analysis of the whole heart ensures optimal representativity The measured enzyme leakage is a function of escape only from myocardial cells via the interstitium to the coronary veins and lymphatics Estimations of elimination rates and distribution volumes are thus not necessary (30) The working rat heart preparation is stable for several hours (18 34) In this study the stability of the preparation is shown for 30 min of anterograde perfusion followed by 30 min of retrograde perfusion demonstrated by the data in Table I Aortic and coronary flows are constant in all groups When the heart rate is kept constant there is a gradual decrease in glycogen concomitant with an increase in myocardial lactate and a de

crease in creatine phosphate When hearts are perfused over a longer period the heart rate decreases unless they are paced

Clamping the ball valve bypass tube induces an almost instant reduction in coronary flow with the hearts still performing the same amount of external work As a result of ischemia there is a subsequent decrease in aortic flow (Fig 3) as described by others (33) In this study the coronary flow decreased to about 25% of normal when ischemia lasted for up to 10 min A further decrease thereafter was probably due to edema and cardiac failure The distribution of myocardial blood flow during ischemia has been extensively studied (23 27 39) It is important to know whether the flow is evenly distributed during ischemia to be able to decide whether myocardial specimens taken for biochemical analysis are truly representative Localized infarctions show various degrees of ischemic damage from the center to the marginal zone (31) which makes it difficult to get comparable samples Different methods for studying regional perfusion have been used including radioactive labelled microspheres (39) There is however a difficulty in getting an even distribution of the spheres ^{3}H antipyrine being inert and lipid soluble has a blood flow limited tissue uptake i e a characteristic that is similar to the noble gases krypton and xenon (26) As can be seen in this study (Figs 7–10) there is an even distribution of flow at the cellular level also during ischemia but because of the reduced flow rate the front of ^{3}H antipyrine has not yet reached the subendocardium at the time of freezing If the heart is frozen at a later time the front is closer to the endocardium However the later the time of freezing the higher is the risk for diffusion of ^{3}H antipyrine Similar studies on the distribution of coronary flow in isolated rat heart by the use of transport of ^{3}O methylglucose and washout of ^{14}C sorbitol indicate an equal distribution of coronary flow in ischemic hearts (35)

The amount of released enzymes has been shown to reflect the severity of ischemic damage (31 40) Ischemia of up to 30 min results in increasing amounts of enzyme leakage suggesting that the amount of this leakage is well correlated to the ischemic damage in this experimental model too After only 5 min of ischemia there is a significant release of ASAT yet ATP is only reduced by approximately 25% and creatine phosphate by 75% which is of the same order as found by others (6 13, 34) Fleckenstein et al (11) have found that the high energy phosphate breakdown becomes critical when the ATP concentration in the myocardium is lowered by about 50% and the creatine phosphate concentration by about 80%

In acute myocardial infarction in man the appearance of enzymes in serum and their maximum levels do not coincide The reasons for this may be different permeability of the cell membrane different volumes of distribution and/or different rates of degradation The deleterious effect of reperfusion of ischemic myocardium has been attributed to bleeding (7) and reoxygenation of the anoxic perfused heart (15) At the end of the ischemic period in this study only a small release of enzymes was detected but a further leakage occurred during the 30 min of reperfusion The question whether enzyme leakage is a sign of irreversible cellular damage is important and has been little discussed It is known that liver cells may leak enzymes without being irreversibly damaged (16 21 28 32) The intermediary coronary syndrome (3 17 24) may well be an example of a similar leakage from the myocardium Ischemia for up to 10 min permits an almost complete restoration of coronary and aortic flow rates after 30 min of reperfusion with a concomitant normalization of myocardial content of glycogen lactate ATP and creatine phosphate However these hearts still show a significant enzyme release It is not likely that the enzyme leakage comes from irreversibly damaged cells as glycogen lactate ATP and creatine phosphate are completely restored to control values This could otherwise only be achieved by supranormal accumulation of the surviving cells and has only been described for glycogen (4)

The results of this study strongly suggest that reversibly damaged cells may leak intracellular enzymes and that small increases of such myocardial enzymes are not necessarily a sign of myocardial infarction

ACKNOWLEDGEMENTS

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Factors Modifying Ischemic Injury in the Isolated Rat Heart

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ABSTRACT The extent of ischemic injury has been studied in the isolated working rat heart utilizing an aortic ball valve that reduces the coronary flow A number of factors were tested including high heart rate, noradrenaline, acidosis alkalosis high afterload β-blockade glucose–insulin–potassium (GIK), palmitate and methylprednisolone Mechanical performance, myocardial contents of ATP, creatine phosphate, glycogen and lactate and the leakage of creatine phosphokinase (CK) from the myocardium to the perfusion buffer were measured and used for determination of the ischemic injury Tachycardia noradrenaline and palmitate are factors that markedly increase the ischemic injury in this preparation GIK and probably metoprolol decrease the release of CK compared with the controls

The introduction of coronary care units decreased the death rate from acute myocardial infarction (AMI) by up to 50% in the early hospital phase (14 27 28 29) This reduction in mortality is mainly due to early detection and treatment of arrhythmias (30 42) There is not much more to be gained from better antiarrhythmic treatment as far as the death rate in the acute phase is concerned as most of the patients now die because of congestive heart failure cardiogenic shock or cardiac rupture (14 27 49) The long term prognosis in myocardial infarction depends to a great extent on the size of the infarction and subsequent heart failure Therefore much interest has been devoted recently to means of reducing the infarction size Clinical trials revealing the beneficial effects of nitroglycerin (5 6 47) phentolamine (1 11) and nitroprusside (9) have recently been reported The beneficial effects of glucose–insulin–potassium (GIK) have been suggested (26 50) as well as rejected (7 39) Much interest has been devoted to the effects of free fatty acids (FFA) on arrhythmias and infarction size (15 31 35) Effects of β stimulation and blockade in the dog have been studied especially by the Braunwald group (23 24 25)

To achieve a further reduction in mortality from AMI it is essential to know what factors significantly influence the ischemic injury and whether their effects can be modified in the clinical situation The purpose of this study was to evaluate the relative importance of such factors

MATERIAL AND METHODS

Animals

Male Wistar rats (350–450 g) were fed a standard pellet diet with water ad lib Twenty minutes after heparinization (2 5 mg i p) the rats were anesthetized with pentobarbital (60 mg/kg b wt i p) The hearts were quickly excised and immersed in ice-chilled isotonic saline

Perfusion technique

After 10 min of retrograde and 2 min of anterograde preperfusion the hearts were made ischemic according to the method described by Neely et al (33) and at the same time subjected to the different conditions named below All hearts were paced at 300 beats/min except for those unpaced After 10 min of ischemia the hearts were reperfused retrogradely for 30 min as described previously (55) Krebs Henseleit bicarbonate buffer was diluted with glucose to give a concentration of 14 mM and gassed with 95% O_2–5% CO_2 at 37°C resulting in pH 7 4 Ascorbic acid (0 1 mg/ml) was added to the recirculating buffer to prevent oxidation of noradrenaline The pH of the buffer was in some experiments varied by changing the concentration of bicarbonate The concentration of sodium ions was adjusted to achieve isotonicity Noradrenaline bitartrate was dissolved in perfusion buffer and 1 ml of the solution was added to the recirculating perfusion buffer in some experiments Metoprolol (Seloken® Hassle Sweden) was dissolved in perfusion buffer and added to the perfusion system to given a final concentration of 10^{-4}M Palmitate 1 5 mM (Sigma Chemical Co St Louis USA)

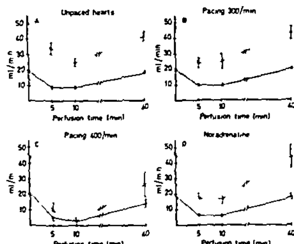


Fig 1 Effects on cardiac output ( ) and coronary flow (—) in hearts ischemic for 10 min and reperfused for 30 min *A* = unpaced (n=7) *B* = paced at 300 beats/min (n=14) *C* = paced at 400 beats/min (n=12) *D* = perfused with noradrenaline 10^{-6}M (n=6) (mean ± S E)

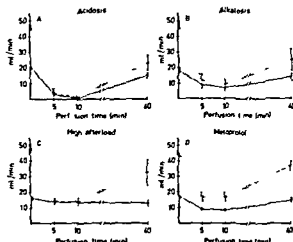


Fig 2 Effects on cardiac output ( ) and coronary flow (—) during 10 min of ischemia and 30 min of reperfusion of isolated hearts when (*A*) perfused at pH 7 1 (n=6) (*B*) at pH 7 8 (n=6) (*C*) with aortic tube clamped (n=6) and (*D*) with metoprolol added 10^{-4}M (n=5) (mean ± S E)

and bovine albumin (Armour Pharmaceutical Co England) were used in the fatty acid experiments Insulin 50 mU/l (Vitrum Sweden) with glucose 500 mg/100 ml and K^+ 10 6 mM were used in the GIK experiments Methylprednisolone (Urbasone® Farbwerke Hoechst AG Frankfurt/M West Germany) was used in a concentration of 30 mg/l

Biochemical analysis

The hearts were powdered in a percussion mortar at the temperature of liquid nitrogen A sample of this powder was taken for biochemical analysis Glycogen was split by amylase and assayed as glucose by the glucose-oxidase method of Saifer and Gerstenfeld (41) Tissue lactate was determined by the enzymatic method of Lundholm et al (22) ATP and creatine phosphate were assayed according to Lamprecht and Trautschold (18)

Myocardial leakage of creatine phosphokinase (CK EC 2 7 3 2) to the buffer measured at the end of the reperfusion period was used as a sign of ischemic cell injury (3 43) CK was assayed in a LKB 8600 Reaction Rate Analyzer (LKB Bromma Sweden) and a standard UV test kit was used (Biochemical Test Combination Boehringer Mannheim GmbH West Germany)

Statistical evaluations were performed according to Student s *t* test and Fisher s permutation test P values ≤0 05 were considered statistically significant

RESULTS

Unpaced hearts or hearts paced at 300 or 400 beats/min (Fig 1*A*–*C*) show a decrease in coronary flow by 50% or more during ischemia The flow is normalized at the end of the reperfusion period Cardiac output also decreases in all three groups of hearts, being most marked in those paced at 400 beats/min and does not return to initial values in the latter One group of hearts was perfused with *noradrenaline 10^{-6}M (Fig 1D) resulting in flow* values comparable with the reference group (paced at 300 beats/min Fig 1*B*) Acidotic perfusion (pH

Table I *Effects on myocardial contents of ATP and creatine phosphate after perfusion under conditions listed below (mean ± S E)*

	Un paced hearts	Hearts paced at 300 beats/min	Hearts paced at 400 beats/min	Nor adrena line (10^{-6} M)	Acidosis (pH 7 1)	Alka losis (pH 7 8)	High after load	Meto-prolol (10^{-4} M)	Glucose–insulin–potassium	Pal mitate (1 5 mM)	Methyl predni solone (30 mg/l)
n	7	14	12	6	6	6	6	5	5	7	6
ATP	21±1	18±3	19±4	17±1	17±1	18±2	19±2	21±1	20±0 5	19±1	22±1
Creatine phosphate	21±3	27±3	23±4	27±2	30±7	21±4	21±2	26±1	27±4	33±1	28±2

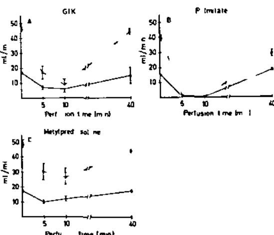


Fig 3 Effects on cardiac output ( ) and coronary flow (—) during 10 min of ischemia and 30 min of reperfusion of isolated hearts when (*A*) perfused with glucose–insulin–potassium (*GIK*) ($n=5$) (*B*) palmitate 1 5 mM ($n=7$) and (*C*) methylprednisolone 30 mg/l ($n=6$) (mean±S E)

7 1) resulted in a marked decrease in the flow rates during ischemia but with good restoration of coronary flow (Fig 2*A*) Alkalotic hearts (pH 7 8) decreased their coronary and aortic flows to the same extent as the controls (Fig 2*B*) Hearts subjected to high afterload (aorta clamped) did not show reduction in coronary flow because of the extremely high perfusion pressure during systole (Fig 2*C*) Perfusion with metoprolol GIK or methylprednisolone did not result in any significant difference in flow rates compared with the controls (Figs 2*D* 3*A* 3*C*) Palmitate added to the perfusion buffer (1 5 mM) resulted in extremely low flow rates during ischemia (Fig 3*B*)

ATP and creatine phosphate were measured at the end of 30 min of reperfusion No significant changes were detected compared with hearts paced at 300 beats/min (Table I)

The range of the values for enzyme activity in the buffer tended to be small for hearts with a moderate ischemic injury As the hearts became more damaged the range of values widened with an increasing number of very high values Fig 4 shows a significant increase in enzyme leakage with increasing heart rate When noradrenaline or palmitate were added to the perfusion buffer a marked increase in enzyme leakage was seen compared with the control group (paced at 300 beats/min) A significantly decreased leakage of CK was found in the group of hearts perfused with GIK added to the buffer No statistically significant differences were registered concerning any of the other factors tested

DISCUSSION

The coronary flow in the isolated rat heart may be reduced to a pre-chosen degree with subsequent normal reperfusion When the metabolic demand exceeds the supply of oxygen substrate and fluid myocardial ischemia results with subsequent deterioration of myocardial function Some hearts rapidly decrease their mechanical activity and tend to be less severely damaged This difference in response to reduced coronary flow explains the rather marked variation in ischemic damage within the group measured as leakage of CK So far only a few of the factors considered to be of importance for ischemic injury have been tested at the same time in one and the same experimental model This makes it difficult to compare the relative importance of different factors

Factors that increase metabolic demand such as tachycardia and high levels of catecholamines seem to be most important for the degree of ischemic injury In the model used in this investigation pacing of the heart is necessary to achieve marked deterioration of mechanical function and metabolism Unpaced hearts often become bradycardial thus decreasing oxygen demand The deleterious effects of an increasing heart rate shown in the present paper are obvious Similar results have been obtained in ischemic dog heart in situ (44)

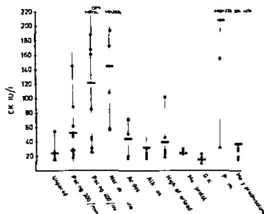


Fig 4 Enzyme activity of creatine phosphokinase (*CK*) in the perfusion buffer under the listed conditions when the hearts had been ischemic for 10 min and thereafter reperfused for 30 min Figures within parentheses are true values Horizontal lines are mean values for each group

Atrial pacing of patients with coronary artery disease can result in lactic acid production as a sign of induced ischemia (4 13 21 38) AMI patients show increased plasma levels of catecholamines (8 12 34) The effects of catecholamines on ischemic myocardial metabolism have been extensively investigated and are indisputably deleterious (23 24 25) The noradrenaline concentration used in this study is rather high but theoretically well within the physiological limits attained when the myocardial stores of noradrenaline are depleted as shown by Wollenberger et al (57) The concentration can be estimated from measurements of total myocardial noradrenaline stores (48)

FFA are believed to increase the risk for cardiac arrhythmias (2, 17 53) and are known to interfere with the metabolism of ischemic myocardium of dog hearts (15, 31) and isolated rat hearts (19) There are probably several reasons for this including a high P/O ratio for FFA transformation of FFA to long chained esters and the accumulation of acyl coenzyme A (37 45 46)

The present study could not confirm the finding of modifying effects of some of the factors tested on the extent of ischemic injury Corticosteroids are known to stabilize lysosomal membranes (20 51) and in high doses to exert peripheral vascular effects (16) that cannot be tested in the isolated perfused heart In dogs subjected to coronary artery ligation some authors have found a marked reduction in infarct size (20 51) but detrimental effects of corticosteroids have been reported by others (40) Acidosis and alkalosis with pH varying from 7 1 to 7 8 probably do not affect cellular metabolism to any great extent but some negative mechanical effects have been reported at pH 7 1 (10 36) At a still lower pH there are marked metabolic and mechanical changes (56) There was no increase in CK leakage which might be due to decreased sensitivity to endogenous noradrenaline and decreased mechanical performance resulting in a low metabolic demand Alkalosis was expected to aggravate the ischemic injury but the higher buffer capacity may have normalized the intracellular pH The effects of different pH levels are complex and need further investigation

High afterload might be expected to cause increased ischemic injury However elevated BP tended to diminish the infarct size in experimental dogs as a result of increased coronary flow (23) In the present study there were no differences in enzyme leakage between the hearts subjected to increased afterload and the controls This is probably due to a fairly well maintained coronary flow because of the extremely high aortic pressure after clamping the aortic tube

The effects of GIK in myocardial infarction have been under debate since the introduction of this therapeutic intervention by Sodi Pallares et al (50) Some authors support this finding (26) but it could not be confirmed by others (7, 39) There are some good theoretical reasons for expecting positive effects of insulin treatment since it lowers the serum FFA level hyperpolarizes the cell membrane and at the same time speeds up glucose transport into the cell The CK leakage was lower in the hearts perfused with GIK in the present study

Beta blockade has well documented effects on the ischemic myocardium of experimental animals (24) as well as of humans (32 52 54) There was a decreased release of CK although not statistically significant from isolated ischemic rat hearts perfused with metoprolol added to the buffer

The question of how to reduce the size of a myocardial infarction has attracted much interest lately It is now recognized that tachycardia high serum levels of catecholamines and FFA are detrimental to the ischemic myocardium and should be avoided in the clinical situation A theoretically optimal therapeutic effect may be obtained by facilitating the metabolism of glucose using GIK lowering serum FFA by adding antilipolytic agents and last but not least reducing metabolic demand by β blockade These therapeutic interventions seem to be too complex for practical applications On the other hand β blockade by itself exerts many of the effects above by reducing myocardial metabolic demand and plasma FFA levels The beneficial effects might be expected to be even more pronounced in intact animals or patients with functioning adipose tissue and adrenals

ACKNOWLEDGEMENTS

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Electrocardiographic Changes in Massive Pulmonary Embolism

I Analysis of the Changes in P Wave and QRS Complex

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ABSTRACT A retrospective analysis of ECG changes during lung embolism has been made in 35 patients (16 male, 19 female) who died of massive lung embolism The presence of lung embolism or infarct was confirmed at autopsy According to the ECG the mean heart rate increased from 91 to 106 The amplitude of the P wave increased in leads I, II III and aVL and decreased in all precordial leads A small increase in the Q wave occurred in leads II III aVF, V_1, V_2 and V_6 Only a small decrease in the R wave occurred in leads I, II and aVL A small increase in the S wave occurred in leads I, II V_1–V_6 when compared with the controls None of the differences was however, statistically significant

A broad spectrum of ECG abnormalities during lung embolism has been described during the last few decades I thas been stated that one or more of the traditional manifestations of acute cor pulmonale ($S_IQ_{III}T_{III}$ RBBB P pulmonale or right axis deviation) occur during lung embolism in only 23% of the cases (7) Others have shown that in the prospective study only 18% of the cases of lung embolism show ECG changes (8) Higher rates have also been presented (4 9) It has been proposed that ventricular dilatation possibly combined with hypoxemia is one factor causing ECG abnormalities during lung embolism (1 7)

Several previous studies deal with the incidence of ECG abnormalities during clinically diagnosed lung embolism The present investigation deals with the quantitative ECG changes during massive lung embolism confirmed by autopsy The changes in the last two ECGs available were compared in order to determine the direction of the changes in pulmonary embolism

STUDY POPULATION AND METHODS

The ECG changes in patients who died of lung embolism in the University Central Hospital of Helsinki (Meilahti) during 1973 and 1974 were analyzed Only cases with the diagnosis of lung embolism confirmed by autopsy were included in the present series which comprises 35 patients (16 male and 19 female) Parameters of the last ECG available before death were measured—by one observer and once in each case—and compared with the control ECG of the same patient registered earlier The mean interval between these two recordings was 140 days (range 1 day–4 years median 11 days)

The following parameters were measured heart rate (calculated from 5 beats) PQ interval (csec) QRS complex (csec) QT interval (csec) electrical axis and amplitudes of P Q R and S waves (mm 10 mm=1 mV) R/S ratio in lead V_1 was also measured Before each registration a calibration was made

Student s *t* test was used in the statistical analysis

RESULTS

When the last ECG was compared with the control ECG (recorded earlier) it was found that heart rate increased from 90 9±3 8 to 106 1±3 9 (S E M) during severe lung embolism The difference was not significant Almost no change was observed in the PQ interval between these two recordings The PQ interval in the control ECG was 15 6 and in the last ECG 15 4 No significant change was observed in the QRS complex (duration) or electrical axis None of the control ECGs showed P pulmonale (≥2 5 mm) whereas it was observed in the last ECG in 3 cases (8 5%) in lead II in 2 cases (5 6%) in lead aVF and in 1 case (3%) in lead III

Changes in P wave

A small increase in the amplitude of the P wave was observed in all extremity leads except for aVF

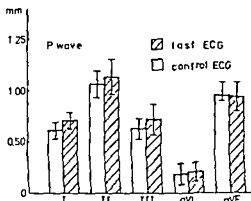


Fig 1 Amplitude of the P wave (±S E M) (10 mm=1 mV) during massive pulmonary embolism The amplitude increased in all extremity leads except for aVF

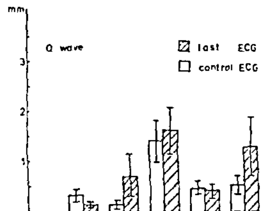


Fig 3 Q wave in extremity leads during massive pulmonary embolism The Q wave increased in the inferior leads and decreased in the others

when the last ECG was compared with the control ECG The changes in detail were from 0 6±0 1 (S E M) in the control ECG to 0 7±0 1 in the last recording in lead I from 1 1±0 1 to 1 1±0 2 in lead II from 0 6±0 1 to 0 7±0 2 in lead III from 0 2±0 1 to 0 2±0 1 in lead aVL from 1 0±0 1 to 0 9±0 1 in lead aVF (Fig 1) The amplitude of the P wave on the other hand diminished in all pre cordial leads The changes were from 0 1±0 1 to 0 0±0 2 in lead V_1 from 0 7±0 1 to 0 5±0 1 in lead V_2 from 0 9±0 1 to 0 7±0 1 in lead V_3 from 0 8±0 1 to 0 7±0 1 in lead V_4 from 0 8±0 1 to 0 6±0 1 in lead V_5 from 0 7±0 1 to 0 6±0 1 in lead V_6 Fig 2)

Changes in Q wave

The Q wave grew in amplitude in leads II III and aVF and decreased in leads I and aVL The detailed changes from the control ECG to the last ECG were from 0 3±0 1 to 0 2±0 1 in lead I from 0 2±0 1 to 0 7±0 4 in lead II from 1 4±0 4 to 1 6±0 5 in lead III from 0 5±0 1 to 0 4±0 2 in aVL from 0 5±0 2 to 1 3±0 6 in lead aVF (Fig 3)

In precordial leads the amplitude of the Q wave increased in V_1 V_2 and V_6 and decreased in the others The changes in detail were from 3 1±1 1 to 3 4±1 0 in lead V_1 from 0 4±0 4 to 1 4±0 8 in lead V_2 from 0 0±0 0 to 0 in lead V_3 from 0 2±0 1 to 0 1±0 0 in lead V_4 from 0 3±0 1 to 0 2±0 1 in V_5 from 0 3±0 1 to 0 4±0 1 in lead V_6 (Fig 4)

Changes in R wave

The amplitude of the R wave was analyzed in all ECGs studied The R wave decreased in all extrem

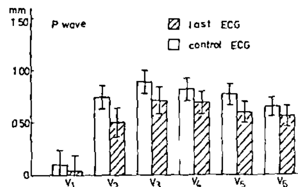


Fig 2 Amplitude of the P wave in precordial leads The amplitude decreased in all precordial leads

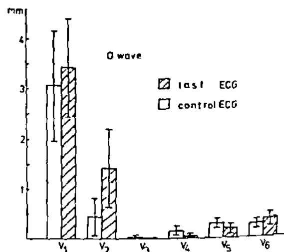


Fig 4 Q wave in precordial leads The Q wave increased in leads V_1 V_2 and V_6 and decreased in the others

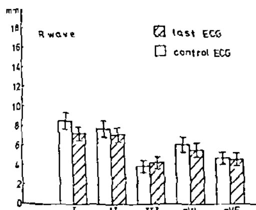


Fig 5 Amplitude of the R wave in extremity leads during massive pulmonary embolism A small increase took place in lead III all the other leads showed a small decrease

ity leads except for lead III where a small increase was registered The specific changes from the control to the last recording were from 8 4±0 8 to 7 2±0 7 in lead I from 7 7±0 8 to 7 1±0 7 in lead II from 3 9±0 6 to 4 2±0 6 in lead III from 6 0±0 8 to 5 2±0 7 in lead aVL from 4 7±0 6 to 4 6±0 6 in lead aVF (Fig 5)

There was almost no change in the R wave in the precordial leads The changes were from 2 0±0 4 to 2 0±0 5 in lead V_1 from 4 8±1 1 to 4 7±0 7 in lead V_2 from 7 7±1 2 to 7 6±0 9 in lead V_3 from 13 5±1 0 to 13 8±1 2 in lead V_4 from 17 0±1 3 to 17 2±1 3 in lead V from 14 3±1 1 to 14 1±1 1 in lead V_6 (Fig 6)

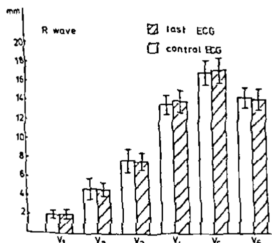


Fig 6 R wave in the precordial leads during massive pulmonary embolism Almost no change was observed

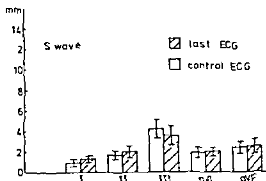


Fig 7 S wave in the extremity leads during massive pulmonary embolism A small increase was observed in leads I and II whereas a small decrease was noticed in lead III

Changes in S wave

A small increase in the S wave was recorded in leads I II aVL and aVF whereas a small decrease was noticed in lead III The changes in detail from the control ECG to the last ECG were from 1 0±0 3 to 1 3±0 4 in lead I from 1 7±0 4 to 2 0±0 6 in lead II from 4 2±0 9 to 3 6±0 9 in lead III from 1 9±0 5 to 2 0±0 4 in lead aVL from 2 4±0 6 to 2 6±0 7 in lead aVF (Fig 7) The corresponding changes in precordial leads were from 11 6±1 2 to 11 6±1 4 in lead V_1 from 15 9±1 4 to 18 3±1 5 in lead V from 14 1±1 2 to 16 5±1 4 in lead V_3 from 10 1±1 2 to 12 3±1 3 in lead V_4 from 5 4±0 9 to 7 9±1 4 in lead V_5 from 1 4±0 3 to 2 6±0 6 in lead V_6 (Fig 8)

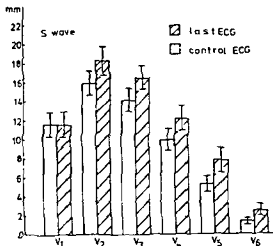


Fig 8 S wave in precordial leads during massive pulmonary embolism A small increase was observed in leads V_2–V_6

The R/S ratio was ≥1 in three control ECGs and in five of the last ECGs RBBB was present in one control and two of the last ECGs An S_IQ_{III} pattern was present in two cases in both control and last ECGs

DISCUSSION

Some of the patients in the present investigation may already have had a sign of lung embolism in their ECG during control registration which took place in many cases only a day before This may in reality improve the significance of the present results

The present results are in agreement with the earlier finding that acute P pulmonale develops in the inferior leads only in some cases (2 7) The increase in the amplitude of the P wave in lead I in addition to leads II and III generally described was observed in the present study The Q wave grew in leads V_5–V_6 due to development of QS complex in some cases as well as in inferior leads II III and aVF which is consistent with the development of the pseudoinfarction pattern described by Stein et al (7)

Almost no change in the R wave was observed in the precordial leads whereas the S wave grew in leads V_5–V_6 This means that the R/S ratio remains almost constant in lead V_1 but diminishes in leads V_5–V_6 This may indicate that no overall hyper of the right ventricle appeared during ssive lung embolism except in two cases This finding is in agreement with observations by others (5 7)

The present results also show that the S wave diminished in lead I The Q wave on the other hand grew a little in lead III These observations show that the S_IQ_{III} pattern described earlier as typical of lung embolism did not occur in the present 35 cases of massive lung embolism

In the present series the R/S ratio in lead V_1 was 1 or more in three control ECGs and increased in two additional cases (5 6%) during massive embolism This observation is in good correlation with the 5–6% incidence of right ventricular hypertrophy during massive pulmonary embolism reported by others (5 7) The conduction distrubances described by Stein et al (7) were not encountered in the present series

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Electrocardiographic Changes in Massive Pulmonary Embolism

II Analysis of the Changes in ST Segment and T Wave

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ABSTRACT A retrospective analysis of the changes in ST segment and T wave has been made in 35 patients (16 male, 19 female) with massive pulmonary embolism in whom the diagnosis was confirmed at autopsy Parameters of the last two ECGs available were measured and compared It was found that during massive pulmonary embolism the most characteristic abnormalities were a decrease in ST segment, which took place in about 70% in leads I and V_5–V_6 Another common change was the inversion of the T wave which took place in 43% in aVF, and in 37% in lead II None of the changes however, was statistically significant

The incidence of ECG abnormalities during pulmonary embolism varies (4 7 8 9) The traditional ECG changes of acute cor pulmonale during pulmonary embolism include $S_IQ_{III}T_{III}$ RBBB P pulmonale or right axis deviation (7)

The most common abnormalities in ECG during pulmonary embolism are changes in ST segment or T wave (1 2 3) These changes are however not specific for lung embolism alone as they may be related to ischemia (7)

The ante mortem diagnosis of pulmonary embolism is difficult (6) In most ECG studies it is made with lung angiography or lung scan The present retrospective investigation deals with the changes in ST segment and T wave in massive lung embolism confirmed by autopsy

STUDY POPULATION AND METHODS

Patients who died during 1973 and 1974 from lung embolism in the University Central Hospital of Helsinki (Meilahti) Finland were included in the present investigation Maximal amplitude deviations (mm 10 mm = 1 mV) of ST segment and T wave from the isoelectric level (PQ segment) were measured from the ECGs in 35 patients (16 male 19 female) in whom the diagnosis of lung embolism was confirmed by autopsy Changes in ST segment and T wave in the last two ECGs available were compared taking the earlier ECG as the control The mean interval between these two recordings was 140 days (range 1 day–4 years median 11 days) The $S_IQ_{III}T_{III}$ index was also calculated (S_I+Q_{III}–T_{III}) A calibration was performed before each registration

Student s *t* test was used in the statistical analysis

RESULTS

A depression of ST segment was the most common ECG abnormality found in 71% in lead I and in 74% in V_5 (Table I) A negative T wave was encountered e g in 43% in lead aVF (Table I)

Changes in ST segment

A small depression of the ST segment was observed between the last ECG and the control ECG in

Table I *Incidence of the changes in ST segment and T wave (%) in 35 patients with massive lung embolism*

	Lead										
	I	II	III	aVL	aVF	V_1	V_2	V_3	V_4	V_5	V_6
ST depression	71	54	31	46	43	6	14	31	54	74	69
ST elevation	0	6	23	9	9	57	40	29	14	3	0
Inversion of T	31	37	26	29	43	29	23	14	29	26	29
Isoelectric T wave	20	20	37	40	17	9	6	3	6	0	11

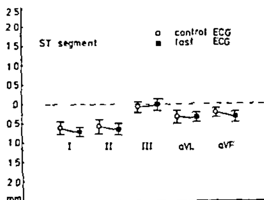


Fig 1 Changes in the ST segment in the extremity leads during massive lung embolism Only a small decrease in the level of ST segment was observed in leads I II aVL and aVF and a slight increase in lead III when the last ECG was compared with the control ECG None of the changes was statistically significant Calibration 10 mm = 1 mV

massive lung embolism in leads I II aVL aVF and V_2–V_6 i e in the inferior and lateral leads (Figs 1 and 2) The changes were from −0 6±0 2 (S E M) in the control ECG to −0 7±0 1 in the last ECG in lead I from −0 6±0 2 to −0 7±0 2 in lead II from −0 1±0 2 to 0 0±0 2 in lead III from −0 3±0 2 to −0 4±0 1 in lead aVL from −0 2±0 1 to −0 3±0 1 in lead aVF (Fig 1)

The corresponding changes in the precordial leads were from 0 6±0 2 to 0 7±0 2 in lead V_1 from 0 6±0 2 to 0 4±0 2 in lead V_2 from 0 2±0 2 to −0 1±0 2 in lead V_3 from −0 6±0 2 to −0 9±0 3 in lead V_4 from −0 9±0 2 to −1 3±0 2 in lead V_5 from −0 9±0 2 to −1 2±0 2 in lead V_6 (Fig 2)

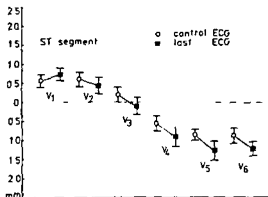


Fig 2 Changes in the ST segment in the precordial leads A small increase in the ST segment was observed in lead V_1 and a decrease in leads V_2–V_6 when the last and the control ECGs were compared

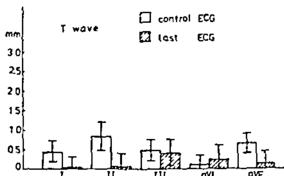


Fig 3 Changes in T wave in extremity leads during massive lung embolism The T wave decreased in leads I II III and aVF and a slight increase was observed in aVL The changes were most pronounced in leads II I and aVF

Changes in T wave

The amplitude of T wave decreased in leads I II III and aVF and a small increase took place in aVL (Fig 3) The changes in detail were from 0 4±0 3 to 0 0±0 2 in lead I from 0 8±0 4 to 0 1±0 4 in lead II from 0 5±0 3 to 0 4±0 3 in lead III from 0 1±0 3 to 0 2±0 4 in lead aVL from 0 7±0 3 to 0 1±0 3 in lead aVF (Fig 3) In the precordial leads the amplitude of T wave increased in leads V_1–V_3 and decreased in leads V_4–V_6 (Fig 4) The changes between the control ECG and the last ECG were from 0 3±0 5 to 1 2±0 5 in lead V_1 from 1 9±0 6 to 2 9±0 6 in lead V_2 from 2 0±0 7 to 2 4±0 6 in lead V_3 from 1 5±0 6 to 1 5±0 6 in lead V_4 from 1 4±0 5 to 1 1±0 6 in lead V_5 from 1 0±0 3 to 0 4±0 4 in lead V_6 (Fig 4)

The $S_I Q_{III} T_{III}$ pattern appeared in only one ECG during massive pulmonary embolism The $S_I Q_{III} T_{III}$

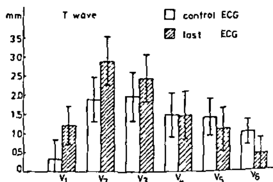


Fig 4 Changes in T wave during massive pulmonary embolism in the precordial leads The amplitude of the T wave increased in leads V_1–V_3 and decreased in the others None of the differences was statistically significant

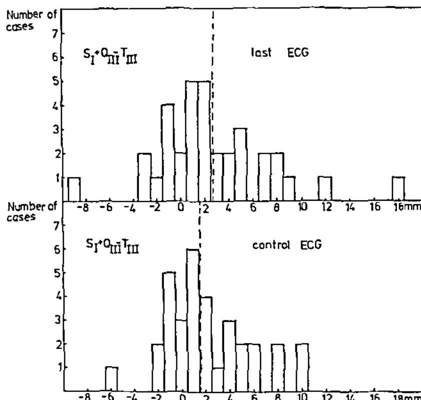


Fig 5 The $S_IQ_{III}T_{III}$ index ($S_I+Q_{III}-T_{III}$) There was only a slight and statistically insignificant increase in the index between the control ECG and the last ECG

index (mm) was also counted from each ECG and a small but statistically insignificant difference was observed (Fig 5)

DISCUSSION

Altough the present results were obtained by different methods the incidence of ECG abnormalities correlates well with the results reported by others (7) The advantage of the present work lies in the accuracy of the diagnosis of lung embolism as confirmed by autopsy

The present results in accordance with those published by others (1 2 3 7) show that typical changes during massive lung embolism were a decrease in the ST segment in the leads of the left side and a small increase in the ST segment of the right side The highest incidence of ECG abnormality in the present series was ST depression which was found in 69–74% in leads I V_5 and V_6 Although ST depression was so common it cannot be judged as diagnostic for lung embolism as it may have other causes e g coronary insufficiency (2) Furthermore it has been shown that the contribution of hypoxia to ECG changes cannot be excluded and that the magnitude of T wave inversion has no correlation to hemodynamic abnormality (7)

According to the present work the amplitude of the T wave decreased in leads I II III aVF V_5 and V_6 Correspondingly an increase in the T wave was observed in aVL and V_1–V_3 The highest incidence of inversion of the T wave took place in lead aVF where a negative T wave was seen in 43% in good agreement with 46% reported earlier

The $S_IQ_{III}T_{III}$ pattern with an incidence of about 16% (7) has been thought to be typical of lung embolism It appeared however in the ECG of only one (3%) of the present patients There was also only a small and insignificant increase in the $S_IQ_{III}T_{III}$ index in the present study

ACKNOWLEDGEMENT

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Complete list of references is given in the preceding article Electrocardiographic changes in massive pulmonary embolism I Analysis of the changes in P wave and QRS complex

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Coffee Consumption and Coronary Heart Disease in Middle-aged Swedish Men

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ABSTRACT The possible association between coffee and myocardial infarction (MI) has been studied both prospectively in a random population sample of Swedish men aged 50 years ($n=834$) and with case control methodology in non selected male patients surviving a MI (age 40–57 years $n=230$) Coffee consumption was significantly associated with two other important risk factors for MI, namely smoking and alcoholic intemperance but weak (non significant) relationships were found with serum cholesterol, serum triglycerides systolic or diastolic BP and dyspnoea on exertion In the prospective study there was no significant relationship between coffee and MI either with univariate or multivariate analysis The retrospectively reported coffee consumption of MI patients was higher than of those who later suffered a MI (the population sample) With the aid of non parametric multivariate analysis of the combined population sample and the series of MI patients a significant association was found between coffee consumption and MI The experience of having had a MI may have affected the patients rating of coffee consumption, or their consumption may have really increased during some months or a few years before the MI

In the search for factors which might be associated with an increased risk of coronary heart disease (CHD) coffee consumption has been studied prospectively (7 12 13) but no significant association was found with CHD The possibility of such a relationship has not attracted much subsequent attention Two case control studies by the Boston Collaborative Drug Surveillance Program (5 17) have shown a higher consumption of coffee among patients surviving a myocardial infarction (MI) compared with patients hospitalized due to other diseases This has led to a series of reviews and comments concerning coffee consumption and CHD (4 6 9 10 11)

The per capita consumption of coffee in Sweden is the highest among reporting countries with a wide range from no coffee to high consumption

A prospective study of a random population sample of men in Göteborg Sweden all aged 50 years when entering the study 12 years ago has given some results concerning the relationship between coffee consumption and CHD In this study age is standardized several variables are studied prospectively and the follow-up is thorough including a high autopsy rate (around 90%) of those dying Univariate and multivariate analysis of risk of CHD has been performed The results have been compared with a non selected series of men of similar age who had survived a MI

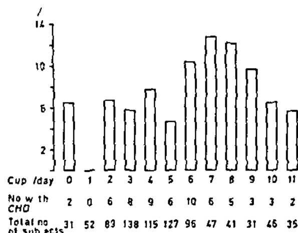
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STUDY POPULATION AND METHODS

Population series

All inhabitants of Sweden have a national registration number that indicates their date of birth and other vital statistics Names addresses and registration numbers are registered by the Official County Census which is continuously updated The sample drawn for the present study 50-year-old men born in 1913 and living in Goteborg was obtained from this register in 1963 All 973 men who fulfilled these criteria and were born on dates divisible by three comprised the study sample Of these 855 (88%) agreed to be examined in 1963 at Sahlgrenska Hospital Göteborg (15)

Seven of the non participants had died four had been admitted to hospital nine did not live in the city at the



F g 1 Inc dence of CHD in the populat on sample of men during 1? years in relat on to coffee consumpt on at age 50

t me of invest gation and 98 refused to partic pate for various reasons mostly because of a negat ve attitude to med cal care The non part c pants had a lower mean in come were more frequently unmarried were more often registered by the Temperance Board and had obta ned s ck allowance to a sl ghtly h gher degree than the par t c pants (14)

Th s prospect ve analys s is based on a study of 834 men Twelve men who had had a clin cal MI and e ght w th ang na pectoris but no MI at the f rst exam nat on in 1963 w excluded from the original group of 855 as well as one (not belong ng to those who later suffered CHD) w mpl te data for the present analys s

A det d cal h story was recorded and cl n cal exam na med throughout the initial study by one of us (G n exam nat on was performed in the morn ng w ects fast ng unt l after blood sam pl ng Deta l n easurements of the variables are presented e The same phys c an checked the average n f cup of coffee wh ch the subjects said they co u d each y The h ghest value within a reported rang ec d

For the stat l smok ng was graded as fol lows never sm k d opped smok ng 2 smoking 1 14 g/day=3 k ng 15–24 g/day=4 smoking≥?5 g/day 5 One c g equal to 1 g of tobacco one cigarillo 2 g and gar 5 g For p pe smokers we used the average numb grams of tobacco cunsumed da ly

Persons experie g dyspnoea when hurry ng on the level or walking mall h ll were scaled as 1 for th s variable others a

Rel able data on nd v dual alcohol consumpt on are very diff cult to obta n from nterv ews In Sweden how ever records of loc d Temperance Boards establ shed by a spec al law prov de an ind cation of alcohol intemper ance The men in the present study who had been re gistered by the Temperance Board at some t me n the r lives were scaled as 1 the others as 0 A l mitation of th s measure is that persons registered for a series of minor offences are in the same group as those with a record of chronic alcohol abuse On the other hand some persons may consume large quant ties without lead ng to publ c offence and registration As our study population was a random sample of the population the same f gure (21%) is val d for the total male population of the same age in Göteborg and probably other large Swed sh cities

The actual levels of casual systol c BP and serum cholesterol were recorded

Hosp tal zat on due to clin cally d agnosed acute MI or fresh CHD at autopsy were used as criteria for the d agnos s of acute CHD The diagnoses were established by the responsible cl n cians or patholog sts Since Nov 1968 all cl n cal cases of MI have been registered in a special register (2) The clinical criteria for MI were those adopted by the Swed sh Society of Card ology central chest pain shock or syncope suggesting an acute MI together with a typ cal transam nase spectrum and/or appearance of a pathological Q wave or localized ST varia tions in the ECG Autopsy criteria for CHD were a fresh myocardial necrosis or a scar or in the absence of any macroscop c scar a total or almost total occlus on of the coronary artery together with a med cal h story suggesting MI or sudden coronary death

Between entry in 1963 and 30th of June 1975 when the mean follow-up time was 12 years 77 subjects had died —24 from acute CHD—and 36 had suffered a non fatal MI

In the population sample un variate and multivariate analyses were performed of risk of CHD for persons with different coffee consumption The relationsh p between coffee consumption and some other factors wh ch ha e been shown to be associated with CHD was also tested by pa rwise analysis of correlation The poss ble add t onal risk caused by increas ng coffee consumption was analyzed by the same type of mult variate log st c model as reported earl er (19)

MI pat ents

During the period Jan 1968–Dec 1970 230 men aged 40–57 years (mean 50) were d scharged al ve from hosp tal after a first MI For 2?0 of them a h story of coffee consumption prior to the MI was recorded about one week after adm ssion The coffee consumption of all men in the populat on sample was recorded in 1963 but for the men w th MI in 1968–70 A b as m ght have been introduced by a time trend n the amount of coffee consumed Therefore we compared the consumption of random population samples of the men of the ame ages in 1963 and 1974 and found the same d stributions Furthermore there was no change in consumption between ages 50 and 59 years n the populat on All the above mentioned variables were measured in exactly the same way in the series of MI pat ents as in the populat on samples Smok ng hab ts prior to MI were evaluated on the bas s of interv ews Accord ng to earl er experience the serum cholesterol has usually returned to pre nfarct on levels about 3 months after an infarct on therefore these values were used (1 3 14)

Student s *t* test was used when comparing results be

Table I *Coffee consumption and incidence of CHD during 12 years in the population sample of men*

Coffee consumption (cups/day)	No of men		Incidence (%)
	CHD cases	Total	
Total series			
0	2	31	6 5
1–5	29	519	5 6
≥6	29	296	9 8
Present smokers			
0	1	6	16 7
1–5	24	275	8 7
≥6	25	191	13 1
Present smokers registered by Temperance Board			
0	1	5	20 0
1–5	7	94	7 4
≥6	12	77	15 6

tween the series of MI patients and the population sample The two series were pooled for the multivariate analysis The multivariate logistic method is not appropriate for analysis of the pooled series A non parametric partial analysis to study the partial correlation was used for the multivariate analysis of the variables In this analysis subgroups of patient were formed for every value of correlated variables and the association between coffee consumption and MI was tested in each subgroup The results from the subgroups were pooled by a special technique described by Mantel (8)

RESULTS

Population series

The univariate analysis of the relationship between coffee consumption and CHD is shown in Fig 1 The percentage of cases with CHD increased somewhat with increasing coffee consumption but the trend is not consistent and does not show any statistically significant relationship

Coffee consumption was significantly associated with two other important risk factors for CHD namely smoking ($r=0\ 22$) and alcoholic intemperance The mean (±S D) coffee consumption of those not registered by the Temperance Board was 4 90±2 79 and of those registered 5 93±3 54 cups a day It was also correlated with physical activity during work ($r=0\ 16$) but as the latter factor was not significantly related to CHD it is possibly of less importance in this context There were very weak (non significant) relationships between coffee consumption and serum cholesterol serum triglycerides systolic or diastolic BP and dyspnoea on exertion

In Table I we have adopted the grouping of individuals into three groups according to coffee consumption which was used by the Boston group (5 17) i e no coffee 1–5 cups a day 6 cups a day or more A grouping was also made with respect to smoking habits and alcoholic intemperance Not even with this type of analysis is it possible to show any association between coffee consumption and CHD This grouping was used in accordance with our primary intention If however the individuals in the two groups 0–5 cups and 6 or more cups a day are compared a significant difference in the incidence of CHD is found between the two consumption groups for the total series but not for the subgroups of present smokers and those registered for intemperance

In a previous paper (19) it was noted that high serum cholesterol high BP smoking dyspnoea on exertion and registration by the Temperance Board each increased the risk of CHD It should be observed that the addition of new variables and/or exclusion of others in the multivariate logistic function changes the coefficients of the other variables and may also influence the level of significance Table II presents the coefficients of the logistic model when coffee consumption is added to the above mentioned variables To be statistically significant zero should not be included in the confidence interval of the coefficient Coffee consumption could not be shown to be significantly related to CHD when smoking and registration by the Temperance Board were included in the analysis Due to the wide confidence interval for β the

Table II *Estimated coefficients of the multivariate logistic model in a random population sample of men*

Variable	Coefficient	95% confidence interval	$p<0\ 05$
Smoking	0 523	±0 330	+
Cholesterol	0 475	±0 316	+
Systolic BP	0 394	±0 265	+
Dyspnoea	0 308	±0.270	+
Registration by Temperance Board	0 220	±0 271	−
Coffee consumption	0 109	±0 300	−

The variable is significantly with CHD if coefficient is separated from zero

Table III *Coffee consumption of subgroups of the population sample and of the series of MI patients*

		Coffee consumption (cups/day)	
	n	Mean	S D
Population sample			
No MI during follow up	786	5 1	3 0
Fatal MI	24	5 9	2 7
Non fatal MI	36	5 5	2 5
Series of MI patients	220	6 5	4 1

β coefficient could vary from −0 20 to +0 40 A slight association between coffee consumption and CHD cannot be discovered without adding more patients to this study When coffee consumption was included alcoholic intemperance lost its significant association with CHD probably due to the slight relationship between coffee consumption and CHD

MI patients and population series

When comparing the MI patients and the population series it was found that the former consumed significantly more coffee than the latter (Table III) This was true both when the patients were compared with those men of the population sample who did not have CHD ($p<0$ 01) and with those who later suffered a non fatal MI ($p<0$ 01) Thus the retrospectively reported consumption (in the series of MI patients) was higher than the consumption reported by those who later suffered MI (in the prospective analysis of the population sample)

With the aid of the non parametric multivariate analysis of the combined population sample and the series of MI patients a significant association ($p<0$ 05) was found between coffee consumption and MI In this analysis we standardized for other variables such as smoking habits serum cholesterol BP and alcoholic intemperance

DISCUSSION

Several factors related to premature CHD seem to be fairly consistent in different prospective studies Some of them are connected with everyday living habits It is possible but far from proven that a change of habits might alter the risk of developing CHD Only preventive trials in man can prove whether such a change of habits can decrease the risk of CHD (18) Many factors have at times been connected with increased risk of not only CHD but other diseases like cancer There seem to be rather few really innocuous habits for people living in the industrialized countries today It is, however important not to incriminate new risk factors without strong evidence at least if the intention is to change these factors

The possible mechanisms by which high coffee consumption might be dangerous for the cardiovascular system are discussed in several of the above mentioned papers It is obvious that caffeine which is a cerebral and cardiac stimulant and possibly also has some effects on the blood lipids might be dangerous to the heart But it defies explanation that tea which contains about as much caffeine per cup as coffee was not associated with an increased risk of CHD in the study by the Boston group (5 17) Tea consumption is fairly low in Sweden (Table IV) and it was not studied prospectively by us

In the present analysis we were not able to find that coffee consumption was a significant risk factor for CHD in a random population sample of middle aged men with strongly varying coffee consump-

Table IV *Per capita consumption of coffee tea alcohol and tobacco in 1964–68 and death rate for CHD in 100 000 males aged 55–64 in 1967 in various countries (ranked according to coffee consumption)*

	Coffee (kg)	Tea (kg)	Total consumption of 100% alcohol (l)	Tobacco (kg)	Death rate for CHD
Sweden	11 93	0 21	5 6	1 62	506
Denmark	10 30	0 32	7 1	3.32	555
Finland	8 77	0 11	4 8	1 35	1 034
Norway	7 81	0 13	3 7	1 47	613
Switzerland	7 48	0 22	10 6	3 54	304
The Netherlands	6 72	0 84	6.2	3 29	564
USA	6 58	0 32	6 3	3 74	929
West Germany	4 85	0 14	12 3	2 50	988
France	4 43	0 06	16 7	1 87	206
Canada	3 85	0 98	7 0	1 88	773
Italy	2 33	0 04	13 4	1 55	300
Austria	2 31	0 09	11 4	1 69	460
New Zealand	1 33	2 87	7 4	2 32	885
Australia	1 28	2 46	8 4	2 24	910
UK	1 07	3 78	6 7	2 39	705

References FAO Trade yearbook and Production yearbook CAN The Swedish Association for Education about Alcohol and Narcotics

tion and this in a situation where we could standardize by a multivariate logistic model for the effect of other risk factors In the present prospective population study we also had the advantage of studying a sample of men of exactly the same age this greatly facilitates analysis and the possibilities of omitting bias due to differences in age which are difficult to omit in practically all other prospective studies The number of those who have reached end points is not large but large enough to show significant associations with all risk factors for CHD which have been found in other prospective studies In spite of having the highest reported coffee consumption the official age specific death rate for CHD is low in Sweden compared with for example Finland United Kingdom or USA (Table IV) This is contrary to the statements by Nichols (11) who had not standardized his death rates for CHD for age When discussing a disease with an evidently multifactorial etiology it is not to be expected that a strong association would appear between the national coffee consumption and CHD

The second part of the present study enabled us to perform a comparison very similar to that of the Boston group (5 17) namely between surviving MI patients and individuals without MI Using a random population sample as controls we could rule out bias due to a possible decrease in coffee consumption because of disease in the controls this cannot be completely excluded in the Boston study where hospitalized patients were used as controls We however made a disturbing finding namely the different consumption values given by the MI patients when interviewed prospectively or retrospectively A plausible explanation is that the psychological trauma of having had a MI has affected the patients rating of coffee consumption or alternatively that the consumption really did increase during some months (or a few years) before the MI If the latter explanation is true some type of psychological and/or physiological change occurring as a premonitory mechanism before a MI might be responsible for the change of habits At present we have no data to corroborate this hypothesis and we know fairly little about spontaneous changes in individual coffee consumption It is possible that coffee has a fairly short effect on the risk of MI and that the increased risk disappears rapidly after a decrease in consumption To be able to show this short term effect in a prospective study the number of cases at risk must be great A study with some possibilities of that kind is under way (18)

Even if some of the results in the Boston study (5 17) and the present study are similar there are also differences The Boston group studied patients with their first as well as recurrent MI The method of grouping consumption may influence the results as shown by a comparison between Fig 1 and Table I Another reason for differences might be the overall risk profile genetic and environmental A risk factor might be more important in a population with higher risk of CHD than in a population with low overall risk Furthermore the non participants of a study might have habits and traits which differ considerably from those of the participants Thus some associations with CHD in a population sample may be hidden e g alcoholic intemperance Conversely coffee consumption or cigarette smoking may seem to be more important if other factors related to coffee and tobacco consumption are not analyzed

Another explanation could lie in different habits of persons with non fatal and fatal CHD As stated earlier in the present report this was not however an explanation in our study

The important risk factors tobacco consumption and alcoholic intemperance were strongly associated with coffee consumption in our study The latter factor might explain some of the relationships found in the other studies The separate factors (high coffee tobacco *and* alcohol consumption) may be additive in some way The positive relationship between the three may also indicate some common psychological background mechanism which could simultaneously increase the risk of CHD and increase the use of coffee tobacco and alcohol

From a biological point of view consumption of excessive amounts of cerebral and cardiac stimulants cannot be recommended but the problem is whether our present knowledge warrants the addition of coffee drinking to the list of dangerous living habits which have to be abandoned According to the present knowledge we do not consider the case against coffee so strong that a ban on coffee consumption can be scientifically justified

ACKNOWLEDGEMENTS

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Sudden Coronary Death in Scandinavia

A Report from Scandinavian Coronary Heart Disease Registers

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ABSTRACT All cases of acute myocardial infarction (AMI) and sudden death due to coronary heart disease have been registered in two Swedish and two Finnish cities during a 1 year period The incidence increased steeply with age in both sexes and was in all age groups far higher in males than in females The age-related incidence in both Finnish cities was definitely higher than in Gothenburg Sweden while the other Swedish register city Boden had an intermediate position The case fatality rate within one year was 44 2% for males and 37 4% for females This difference was already apparent in the first hour after the onset of the attack when the case fatality rate was 15 5% for males and 9 3% for females A history of previous cardiovascular disease was much more common in AMI patients than in the general population It was also slightly more common in subjects who died suddenly than in AMI patients who did not die suddenly

Discussions on the establishment of community based Coronary Heart Disease Registers as one step in a cooperative campaign against ischemic heart disease (IHD) were initiated in Europe in the late 1960s It was thought that these registers would provide a basis for collaboration with public health authorities in preventive cardiovascular programs in addition to giving opportunities for the study of current medical care The Regional Office for Europe of the World Health Organization agreed to act as coordinator for the registers and a coordinating center was subsequently established in Geneva Switzerland

Seventeen centers from European countries joined the program (Fig 1) Four were from Scandinavia five from other countries in Western Europe and eight from Eastern Europe Two centers outside Europe—in Tel Aviv Israel and in Perth Australia—also contributed data to the study The first common report on this collaborative study has been published recently (12) The purpose of this report is to point out some differences found between the Scandinavian registers

STUDY POPULATION AND METHODS

The total population base in four Scandinavian registry cities Gothenburg and Boden in Sweden and Helsinki and Tampere in Finland was 1 150000 persons The [illegible] population [illegible] men and women aged [illegible] 64 was 7 5600 according to the census [illegible] Table I which gives the percentage [illegible] Scandinavian register [illegible] was [illegible] in the Swedish [illegible]

A person was registered [illegible] (AMI) [illegible] AMI [illegible]

The Coronary Disease Registers were established under the auspices of the Regional Office for Europe of the WHO Copenhagen Denmark The [illegible] the WHO Geneva Switzerland

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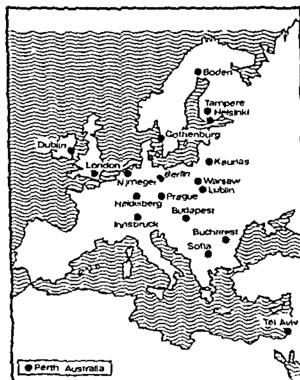


Fig 1 WHO Ischemic Heart Disease Registers (1971)

Another important category to be registered were patients who died before being seen by a physician (medically unattended deaths) Full coverage of these cases was achieved through close liason with the medico-legal authorities

An initial record form was completed in all registered cases as soon as possible after the onset of the episode Information about fatal cases was usually obtained from family members relatives or friends The first review record form was filled in four weeks after the onset of symptoms or earlier if the patient was discharged from *hospital or died prior to that At this time all information* about clinical symptoms ECG serum enzyme levels and post mortem examination was reviewed and the patient was allocated to one of the following exclusive categories (6) definite AMI possible AMI fatal case with insufficient data no AMI The criteria for the categories were as follows

Definite AMI Living patients with unequivocal serial ECG changes or typical history of chest pain and elevated serum enzyme levels or equivocal ECG changes and elevated enzymes Fatal cases with naked eye appearance of *fresh myocardial infarction and/or recent coronary occlu*sion found at necropsy

Possible AMI Living patients with typical chest pain but insufficient evidence to include the case under definite AMI and no good evidence for *another diagnosis Fatal* cases with typical chest pain or clinical or autopsy evidence of chronic IHD when the evidence was insufficient to allocate to definite AMI and there was no good evidence for another cause of death

Fatal case with insufficient data Cases with no autopsy no history of pain no previous history of chronic coronary heart disease (CHD) and no other diagnosis

No AMI Living patients with equivocal ECG changes which were not supported by history of chest pain or elevated serum enzymes or when chest pain was explained by another diagnosis Fatal cases when another diagnosis was made clinically or at autopsy

The autopsy rate for patients in this report was 82 9% *Only one case fell into the category insufficient data All* registered patients were followed up for one year after the onset of registered episode or until death The patients who had more than one event (5 6%) have consequently been recorded more than once in the register However readmission to the register was not possible within 28 days of a previous episode 2 8% of the registered patients did in fact suffer a reinfarction during that period

The completeness of the registers was evaluated in different ways Hospital discharge records from all departments were checked for the diagnosis of AMI in all hospi*tals within the register area after the end of the registration* period The death certificates were also rechecked after the registration period Although it is obvious that silent non fatal AMI will not be detected with this study design rare cases were picked up from routine ECG records

RESULTS

The annual incidence of definite and possible AMI per 1 000 persons in relation to age and sex is shown *in Table II The proportion of definite possible* AMI was 3 1 The rapidly increasing incidence of AMI with advancing age is clearly evident as is the far higher incidence of males compared with females The female incidence seems to reach the male rate with a delay of approximately 15 years *The age related male incidences in the two Finnish* centers are far higher than in Gothenburg while Boden has an intermediate position

The percentage fatality rates versus time after onset of the attack for men and women are given in Table III The sudden death rate (1 hour case fatality rate) is 65% higher for men than women No information about the duration of the fatal attack was available for 5–6% of the fatal cases Most of

Table I *Composition of the study population (1971)*

	Population aged 20–64	Age distribution (%)			
		20–39	40–49	50–59	60–64
Gothenburg	274 830	47 2	21 8	21 5	9 5
Boden	22 380	45 8	21 5	22 4	10 3
Helsinki	332 120	55 2	18 8	17 4	8 7
Tampere	96 270	54 4	20 1	17 3	8 2
	725 600				

Table II *Annual incidence of acute myocardial infarction per 1 000 persons by age and sex in 1971*

Center	Age group 20–39	40–44	45–49	50–54	55–59	60–64	20–64
Males							
Gothenburg	0 1	1 0	3 2	5 5	8 1	9 7	2 6
Boden	0 2	2 6	3 3	9 0	12 7	16 0	4 1
Helsinki	0 4	4 8	9 3	14 5	21 8	26 9	7 3
Tampere	0 2	4 4	6 8	13 3	16 2	25 4	6 2
Females							
Gothenburg	0 03	0 2	0 3	0 9	2 0	3 1	0 6
Boden	0	0	2 4	3 1	6 5	2 7	1 4
Helsinki	0 1	0 4	1 7	2 4	4 3	8 6	1 6
Tampere	0	0	0 7	1 9	2 6	7 1	1 1

these deaths occurred before admission to hospital and it is likely that the majority were sudden Therefore the given percentages probably underestimate the real proportion of sudden deaths The sex difference in 1 year case fatality rate is about 6% This difference was already apparent in the first hour after onset of an episode There is no sex difference in case fatality rate in myocardial infarction during the period of one hour to one year after onset

The distribution of time of death over the day as well as over season of the year was homogeneous Of all sudden deaths 91% occurred outside hospital More than half of the non hospital deaths (52 1%) were witnessed one third (34 5%) not witnessed and in 13 4% this information was missing The attack occurred in most cases (60 8%) at home and in only 9 1% at work

Information on various characteristics of the registered patients who died suddenly have been collected but are not yet available Data characterizing survivors of an AMI compared with the general population have been published from Gothenburg (2 3) and data on sudden deaths compared with non fatal AMI are available from Helsinki (14)

A history of cardiovascular disease—such as previous AMI angina pectoris dyspnea upon exertion intermittent claudication hypertension and stroke—was much more common among the AMI patients than among the general population Diabetes gallstones renal calculi and chronic bronchitis but not peptic ulcer were also overrepresented among the AMI patients (2)

The patients were also characterized by a higher proportion of smokers and of physically inactive persons both during leisure and work A high degree of mental stress prior to AMI was also more common than expected according to data from the general population A record of intemperance was equally common among survivors and the general population but was more common among the cases of sudden death (3) This association with intemperance has also been found in a prospective study from Gothenburg (17)

A history of previous cardiovascular diseases was slightly more common in subjects who died suddenly than in the AMI patients not dying suddenly (14) Seventy four per cent of the males and 56% of the females who died suddenly had had symptomatic CHD before death 38% of the males and 32% of the females had previously experienced a verified myocardial infarction 30% of the males and 50% of the females had had a diagnosed arterial hypertension while 9% of the males and 15% of the females had had diabetes mellitus Only 13% of the males and 12% of the females among those who died suddenly had had no cardiovascular disease diagnosed before the fatal attack

Table III *Percentage fatality within 1971 after the onset of the attack by sex*

	Time interval Not known	≤1 h	1–24 h	24 h–1 y	Total 1 year mortality
Males (n = 1 649)	6 0	15 5	8 4	14 3	44 2
Females (n – 503)	5 0	9 3	8 8	14 3	37 4

The prevalence of smoking was similar in AMI patients who died suddenly and in those who did not On the other hand when the smoking habits were further analyzed it appeared that heavy smoking was much more common in the sudden death group than in the AMI patients Hypertension was also more often associated with sudden death than with AMI Relative weight physical activity social class or marital status did not markedly contribute to the characterization of the sudden death patients On the other hand psychosocial factors seemed to be important precipitating factors for sudden death (13)

Premonitory symptoms during four weeks preceding the attack were recorded in two-thirds of the AMI patients in the Helsinki study The percentage was a little lower 53% in the sudden death group but this kind of information is probably underestimated as it was obtained from relatives Half of the persons who died suddenly had seen their doctor during the preceding month and one fourth during the week preceding the fatal attack

DISCUSSION

Relatively few epidemiological studies have been reported on the occurrence of AMI or sudden death in a whole community While vital statistics have provided information about mortality in CHD in different areas their reliability may vary and data based only on death certificates are often too inaccurate to estimate the sudden death rate The establishment of WHO Coronary Heart Disease Registers was a step forward in this respect and has provided valuable and more complete information about AMI in 17 European communities

The comparability of data collected by Scandinavian registers is probably high The hospital and public health systems in Scandinavia are rather uniform and data were collected using standardized forms Most AMI patients are treated in a few big hospitals within their area of residence Hospital records were checked almost daily for the present study All deaths outside hospital must be reported by law to the police and fatal cases could be registered very soon after death Completeness of registration was checked afterwards in several ways and seemed to be very high probably higher than 95% The high autopsy rate 82 9% is a guarantee of diagnostic accuracy in fatal cases which particularly in sudden deaths is of crucial importance On the other hand, the category possible AMI no doubt includes false positive survivors who were admitted only on the basis of acute retrosternal pain lasting for at least 20 min This possible bias may be avoided by accepting only cases which fulfill the criteria of definite AMI

A high incidence of AMI in Finland was found in the register study High mortality in CHD has been reported earlier in vital statistics and in the Seven Countries Study (7) The higher incidence of AMI in Boden North Sweden compared with Gothenburg Southwest Sweden is also supported by vital statistics although there has so far been no explanation of this difference

The sudden death rate in relation to total CHD mortality in Scandinavia for men and women is similar to that reported from the US (1 5 8 9 15) and UK (4 10) both countries with high incidence of CHD

One of the purposes of the WHO register study was to contribute to programs in Europe for prevention and control of CHD Some centers have already initiated community based preventive trials (11 16) At these centers the registers might be reopened for sample periods to assess any changes in the incidence of AMI in the community which may have occurred in the interim

In addition WHO working groups have made proposals for further studies linked to the CHD registers for example evaluation of the role of mobile coronary care units and rehabilitation programs for myocardial infarction patients and the natural history of the CHD with regard to prodromal symptoms of myocardial infarction and sudden death

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Oesophageal Acid Perfusion Test as a Complement to Work Test in Patients with Chest Pain

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ABSTRACT Out of 121 patients referred to a work test on account of chest pain 67 with a case history typical for oesophageal dysfunction have been further investigated with an acid perfusion test during continuous ECG monitoring Neither arrhythmias nor ST T changes were induced by the procedure Of the 67 patients 6 had a "positive related" acid perfusion test 23 a "positive unrelated" and the remainder a negative test Five of the 14 patients with pathological effort ECG belonged to the group with a positive acid perfusion test The acid perfusion test, as a safe routine in close connection with the effort ECG in selected patients is a useful technique for establishing whether or not the patient's symptoms have an oesophageal component

In Sweden the diagnosis of chest pain of unknown origin often includes a work performance test which is usually performed in a Department of Clinical Physiology At our hospital approximately half of the patients are referred directly from general practitioners and the remainder from the Department of Medicine (including Cardiology) We recently made a separate investigation with oesophageal manometry including an acid perfusion test of a group of such patients and found an incidence of oesophageal dysfunction of 71% (16) For this reason we wanted to add a simple oesophageal function test which could be performed shortly after the work test Oesophageal manometry is rather time-consuming and the patient is obliged to make an additional (out patient) visit to the hospital Therefore the acid perfusion test of Bernstein and Baker (4) seemed more suitable for the present purpose as it does not require complicated apparatus and takes one hour at most to perform

Thus one aim of the present paper is to test whether the use of the acid perfusion test shortly after the work performance test is a practical way of getting a more definite diagnosis during a single hospital visit Since oesophageal irritation may induce ECG changes in patients with ischaemic heart disease (IHD) (9) a second aim of this paper is to investigate whether oesophageal acid perfusion induces ECG changes in this patient group

SUBJECTS AND METHODS

One hundred and twenty-one male patients referred to a routine work performance test for chest pain were asked to fill in a questionnaire aimed at detecting oesophageal dysfunction (14) whilst awaiting the work test Eighty five patients had a positive questionnaire and were asked to partake in an acid perfusion test Sixty seven patients with a mean age of 52 years (range 21-69) joined the investigation The ECG findings at rest are given in Table I

The work performance test was undertaken on an electrically braked bicycle ergometer with a stepwise increase of the load every 6th min The ECG was registered recumbent before and 1 2 4 and 10 min after the test (extremity+V_{1-6} leads) and continuously during work using 6 precordial leads with the reference electrode on the forehead ECG changes at the work test were classified according to Areskog et al (1) ST changes during work were scaled from 0 to 3 where 0 denotes no change outside ±1 mm and 3 denotes a junctional ST depression of 3 mm or more and form change T wave changes after work were scaled from 0 to 3 where 0 denotes no or moderate flattening of T wave and 3 denotes a transient depression of T wave to more than 2 mm negative within 2–4 min after work The sum of the ST and T wave changes were calculated A typical ECG for coronary insufficiency— pathological effort ECG —was defined as 2–6 points on the above scale Of 4 patients receiving digitalis therapy none had pathological effort ECG ECG changes at rest were classified according to the Minnesota Code (11) Chest pain in connection with the work test

Table 1 *Findings on ECG at rest*

	Minnesota Code	Acid perfusion test Positive related	Positive unrelated	Negative
Normal ECG	0	2	18	23[b]
St p infarction	1–1		1	1
High QRS amplitude	3–1	2	2	3[a]
ST depression	4		1	5
Negative T waves	5	2		3
Intraventricular conduction defect	7		1[a]	3
Total		6	23	38

[a] 1 digitalized patient [b] 2 digitalized patients

was classified by a physician with consideration to character site and time course according to Areskog et al (1)

The acid perfusion test was started about half an hour after the work test 0 1 M HCl was infused at a rate of approximately 5 ml/min into the distal oesophagus If the patient experienced pain or substernal discomfort the solution was changed without his knowledge to 5 5% glucose until the pain or burning subsided The acid infusion was then restarted and the test was considered positive if the "pain could be reproduced 3 times within half an hour If the patient stated that the pain was the same or similar to that which made him call upon his doctor the response was regarded as positive related (4) Positive unrelated responses were those in which the acid perfusion repeatedly induced oesophageal symptoms that were totally unlike the clinical symptoms which had brought him to the work performance test The test was considered negative if pain or substernal discomfort was not provoked within 30 min of acid infusion

A 12 lead ECG was recorded continuously during the acid perfusion test with a paper speed of 10 mm/sec for detection of arrhythmia and whenever the patient complained of chest discomfort with a paper speed of 50 mm/sec for analysis of ST T changes

Student s *t* test for paired samples was used for statistical analysis

RESULTS

Six patients had a 'positive related response to the acid perfusion test i e the discomfort provoked at acid infusion was the same ($n=1$) or similar to that ($n=5$) which had made the patient request medical attention (Fig 1) All 6 subjects described the discomfort as a burning sensation behind the lower part of the sternum and 3 experienced heart burn as well

The patient whose pain during the acid provoking test was exactly the same which had brought him to the investigation was a 44-year old male with a normal ECG at rest and during work and without chest pain at the work test Two of the patients with similar pain at the acid perfusion test perceived the same pain at the work test but both patients

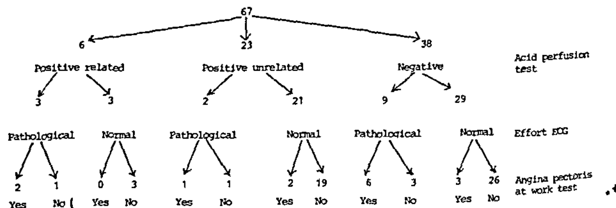


Fig 1 Results of acid perfusion test effort ECG and perceived chest pain at work test in 67 patients investigated with acid perfusion test

al o had ECG s gns of coronary insufficiency at the work test Of the remain ng three pat ents with posi tive related acid perfus on tests none perceived chest pain at the work test one had an abnormal effort ECG and two had a normal ECG at rest and during work

Twenty three pat ents had pos tive unr lated tests i e the oesophageal symptoms produced were d fferent from the r clinical symptoms (Fig 1) The d scomfort was expressed as heart burn by 7 pat ents as a burn ng sensat on beh nd the lower part of the sternum by 2 and as a comb nat on of these symptoms by 12 One pat ent experienced a burn ng sensat on in the ep gastrium and one a right s ded thoracic pa n One of the 23 pat ent had ECG s gns of coronary insufficiency at the work test and one of an old anterior infarction

Th rty eight pat ents had a negative ac d perfu s on test n ne of whom had ECG s gns of coronary nsuff c ency at the work test and one of an old infarct on on ECG at re

The heart rate immed ately after placement of the tube was 83+13 (S D) beats/m n in the pat ent group w th posit ve tests and 84+17 n the group with negat ve tests The heart rate decreased sl ghtly but s gn f cantly towards the end of the test by 9+5 (S D) in the pos t ve group and by 7+10 n the negative group The heart rate did not change s gn f cantly n connect on w th pa n n the pos t ve groups

ST T changes were not noted on any occasion in any of the groups in connect on w th the ac d perfu s on test Arrhythm a (premature beats) v as noted in a total of 7 pat ents n the group with negative tests and in none in the pos tive groups All 7 pa t ents w th arrhythm a had arrhythm a both before and after the test and also n connect on w th the wo k performance test

DISCUSSION

One of the reasons for referring pat ents w th a h story of effort related chest pain to a ork performance test is to establ sh or reject the d agnos of card ac d sease We have recently shown that such pat ents have a h gh inc dence of oesophageal dysfunct on (16) It therefore seemed log cal also to invest gate the oesophagus n close connect on to the work test For pract cal reasons a s mple procedure l ke the ac d perfus on test wh ch can be performed shortly after the work performance test should be ideal for th s purpose The value of this test in the d agnos s of oesoph ageal pain is well established (? 3 4 5 1? 13) Oesophageal stimulat on as produced by balloon d stent on of the oesophagus may however cause arrhythm as and ST T changes on ECG (9) Such changes are also induced in connection with gastroscopy (6 7 10) Bennett and Atk nson (3) reported that one out of 11 studied patients with coronary insufficiency had ischaem c ECG changes in connection with oesophageal ac d perfus on We therefore felt that the ECG response to the acid perfusion had to be invest gated in th s patient group before a more general use of the test could be advocated

In contrast to the above invest gations no arrhythm a or ST T changes were induced by our test procedure In the fe v pat ents v ith arrhythmia before the test the arrhythm a was not aggravated We have also performed the ac d perfus on test in about 70 other pat ents with proven IHD but with out ECG surveillance and without untoward effect (unpubl shed results) It therefore seems that myocard al ischaemia or arrhythm a is not a com mon feature of the acid perfus on test and the ex istence of IHD does not contraind cate its use

The inc dence of positive tests in our material is h gh To our knowledge no randomly selected popu lat on material of acid perfus on tests exists but Behar et al (2) investigated 20 age matched normal subjects 3 of whom had a pos tive test S egel and Hendrix (12) reported that not a s ngle pat ent n the r control group (*n* ?5) had a pos tive test while 24 out of ?5 patients with the cl n cal diagnos s of oesophag t s had pos t ve tests Kappeler et al (8) found that 2 out of 13 normals had a pos t ve test It therefore seems unl kely that the h gh ncidence of pos tive tests in our material reflects a h gh inci dence n the populat on as whole It is more likely that th s pattern is conf ned to pat ents with chest pa n In a randomly selected group of subjects in vest gated by oesophageal manometry but not by ac d perfus on test (14) the inc dence of oesoph ageal dysfunct on was lower (14%) In another patient populat on of 168 pat ents w th pos tive (re lated or unrelated) ac d perfus on tests 73% had a h story of chest pa n and 80% pathological oesoph ageal manometry (16)

Cons dering this the obvious quest on is whether it is worth vh le from a d agnost c po nt of v e v to use the ac d perfus on test liberally in connect on

with diagnostic work tests Six of the 67 tested patients had a positive related acid perfusion test while 14 had a pathological effort ECG Twenty three had a positive unrelated test Thus even if only the positive related tests are taken into account the diagnostic yield of the acid perfusion test under these circumstances is certainly high enough to consider the use of the test in selected patients

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The Effect of Clofibrate on Glucose Tolerance, Insulin Secretion, Triglycerides and Fibrinogen in Patients with Coronary Heart Disease

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ABSTRACT. The effects of clofibrate treatment have been monitored in a double blind cross-over study conducted in 16 male patients with coronary heart disease. Most had latent diabetes mellitus with elevated and delayed insulin release after i.v. glucose administration. Blood glucose and insulin levels were measured during repeated i.v. glucose tolerance tests in each patient and serum triglyceride and plasma fibrinogen were estimated at intervals. Clofibrate treatment significantly lowered fasting blood glucose levels ($p<0.01$) and improved the glucose tolerance ($p<0.01$). Fasting plasma insulin levels and those at 30 min after glucose loading were reduced ($p<0.05$). Serum triglycerides ($p<0.001$) and plasma fibrinogen levels ($p<0.05$) were lowered during the treatment period. The change in k value (glucose utilization) did not correlate to changes in triglyceride or fibrinogen. This study confirms the beneficial effect of clofibrate therapy on abnormal glucose tolerance observed by other workers. It is suggested that clofibrate acts by reducing peripheral insulin resistance.

The role of clofibrate in the prophylaxis and treatment of coronary heart disease (CHD) remains controversial. The coronary drug project (4) did not demonstrate any effect of clofibrate therapy on morbidity and mortality from CHD, whereas several other trials indicated that clofibrate improves the prognosis in groups of patients (2, 17, 21). The possible beneficial effect of clofibrate may not be solely related to its lipid lowering action (6). It has been shown to reduce plasma fibrinogen levels (3) and to have effects on glucose and insulin levels (11, 12, 19, 20, 23, 24). Glucose intolerance and deranged insulin secretion are frequently found in patients with CHD (1, 8, 10, 16, 22). Also secondary hypertriglyceridemia is often seen in diabetic patients (13) and may be associated with their increased incidence of CHD. These risk factors have been evaluated in this cross-over study of clofibrate and placebo in patients with CHD and impaired glucose tolerance.

MATERIAL

Eighteen men were entered into the study. They had all had a myocardial infarct 6–18 months previously. During the study period one patient suffered a reinfarction and was excluded. One patient complained of dyspepsia on clofibrate treatment and was withdrawn. The remaining 16 patients were 41–67 years old (mean 58). Thirteen patients had a sufficient i.v. glucose tolerance test (GTT) disturbance to merit a diagnosis of latent diabetes. Three had low and 10 elevated and delayed insulin release after glucose loading. Fourteen were smokers and 8 were more than 15% above their ideal body weight. Slight hypertension (untreated) was noted in four. Seven patients had hyperlipoproteinemia: two type II*a*, one type II*b* and four type IV (13).

METHODS

A total of 144 non-diabetic men diagnosed as having a myocardial infarct were subjected to an oral GTT two weeks after the infarct. After an overnight fast, each patient was given oral glucose in a dose of 1 g/kg b.wt. Capillary blood samples were taken for glucose estimation fasting, 2 and 2½ hours after the glucose ingestion. In 36 of the patients, two of the following three glucose estimations were above the stated levels: fasting more than 100 mg/100 ml, after 2 hours more than 140 mg/100 ml, after 2½ hours more than 120 mg/100 ml. Of these men 18 agreed to enter the trial. They received clofibrate (Atromid-S) 2 g daily or matching placebo, using a randomized double-blind technique, for 12 weeks. Thereafter they were studied for a further 12 week period with a change of therapy from placebo to clofibrate or vice versa, without compromising their double-blind status. Cl [illegible] placebo capsules

Table I *Mean baseline and posttreatment values after 4 8 and 12 weeks on clofibrate or placebo in patients*

	Weeks of treatment						
	Baseline	Clofibrate			Placebo		
	0	4	8	12	4	8	12
Area under the glucose tolerance curve	235 2	214 1	216 9	214 2	229 9	236 1	2?6 8
Fasting glucose (mg/100 ml)	99 6	89 9	93 9	90 7	97 1	102 8	103 8
K value (% glucose elimination/min)	1 18	1 38	1 42	1 33	1 32	1 15	0 99
Area under the insulin curve[b]	30 0	27 9	22 3	25 6	35 9	24 9	24 9
Log_{10} triglycerides (mg/100 ml)	2 021	1 935	1 939	1 925	2 055	2 035	2 103
Fibrinogen (mg/100 ml)	342 5	334 4	292 5	289 4	351 9	345 6	348 8

[a] Adjusted for the unequal number of patients in the two sequences of treatment
[b] Based on 15 patients due to missing values
Significance of the difference *$p<0\ 05$ **$p<0\ 01$ ***$p<0\ 001$

were supplied by ICI The patients were encouraged not to change their dietary or activity habits during the trial period Examinations were carried out after an overnight fast before the commencement of the study and thereafter every 4 weeks At each examination venous samples were drawn in a standardized way for triglyceride (7) and fibrinogen (5) estimations The fibrinogen method gives 6% lower results than immunodiffusion (Partigen Behringwerke $r=0\ 89$ $n=23$) On each occasion an infusion of 25% glucose (0 5 g/kg b wt) was given over a 5 minute period and samples were taken for blood glucose and insulin before and after 10 30 45 and 60 min The k value (% glucose elimination/min) was calculated with a microcomputer from 30 45 and 60 min glucose values Blood glucose was measured by the orthotoluidin method (15) Immunoreactive insulin was determined on frozen heparin plasma samples using the Phadebas insulin test (25) all samples from each patient being examined for insulin in the same series

RESULTS

Of the 16 patients who completed the treatment periods 7 were treated with clofibrate for 12 weeks prior to placebo and 9 in the reverse order The technique of multiple linear regression has been applied only to the data collected after 12 weeks of treatment for six parameters triglycerides fibrinogen fasting glucose k value and areas under the glucose tolerance and the insulin curves In addition a similar analysis has been carried out on the insulin data comparing treatment effects on fasting levels and those at 10 30 45 and 60 min after i v glucose Adjusted means presented in Tables I and II allow for the unequal number of patients in the two sequence types Table I shows mean levels throughout the study for all parameters Adjusted means after 12 weeks of treatment are given with the difference in means and its standard error Table II shows fasting insulin levels and those at 10 30 45 and 60 min after i v glucose Fig 1 illustrates the behaviour of means for the six parameters and also for fasting insulin levels and those measured 60 min after a glucose load Each graph shows two plots—one for those receiving clofibrate before placebo and the second for those receiving placebo prior to clofibrate

Glucose tolerance (Table I Fig 1)

After 12 weeks of treatment with clofibrate the average fasting glucose level of 90 2 mg/100 ml was

Table II *Adjusted insulin means after 12 weeks of treatment*

	Clofibrate	Placebo	Difference in means	S E of the difference
Fasting	11 0	15 0	4 0*	1 58
After glucose (min)				
10	25 4	27 1	1 7	2 28
30	25 2	29 3	4 1*	2 32
45	27 8	29 8	2 0	2 83
60	26 3	29 2	2 9	2 69

*$p<0\ 05$

justed means after weeks			
ofibrate	Placebo	Difference in means	S E of difference
3 5	227 5	14 0	9 49
0 2	104 3	14 1*	4 86
1 34	0 99	−0 35**	0 094
5 1	27 7	2 6	2 08
1 918	2 110	0 192***	0 045
?0	346 1	54 1*	20 97

significantly lower ($p<0\ 01$) and the k value of 1 34 significantly higher ($p<0\ 01$) than the corresponding placebo means of 104 3 and 0 99 respectively Although there was a reduction in mean area under the glucose tolerance curve on clofibrate this was not sufficient to be statistically significant

Insulin secretion (Tables I and II Fig 1)

Mean insulin levels measured after fasting and up to 60 min after glucose load are shown in Table II At each point the clofibrate mean is lower than the placebo mean and significantly lower after fasting and 30 min after an i v glucose load ($p<0\ 05$) Mean areas under the insulin curve have also been calculated and are given in Table I but the difference in treatment means was not statistically significant

Triglycerides (Table I Fig 1)

The triglyceride data were not normally distributed and the analysis is therefore based on $\log_{10}$ transformed data The difference in treatment means is highly significant ($p<0\ 001$) The adjusted clofibrate mean value is 83 mg/100 ml on the untransformed scale compared with the placebo mean of 129 mg/100 ml

Fibrinogen (Table I Fig 1)

The mean fibrinogen level after 12 weeks of clofibrate treatment was significantly lower than the placebo mean ($p<0\ 05$)

Correlations between changes in glucose elimination and triglyceride or fibrinogen levels

Correlation coefficients have been calculated between changes in k value and changes in the triglyceride and fibrinogen levels the changes being the differences in pretreatment or 12 week placebo figures compared with those at 12 weeks on clofibrate None of the correlation coefficients was statistically significant

DISCUSSION

Diabetes and disturbed insulin release are probably associated with the development of CHD (1 8) Clofibrate has been used for the prophylaxis and

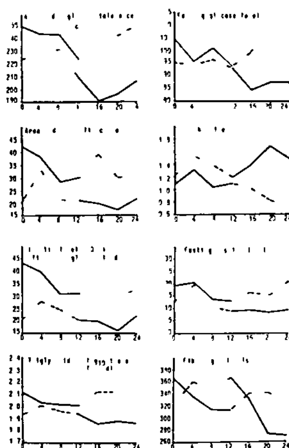


Fig 1 Mean values in 7 patients during treatment with clofibrate prior to placebo (—) and in 9 during treatment with placebo prior to clofibrate ( ) Glucose triglycerides fibrinogen mg/100 ml insulin μU/ml The areas under the glucose and insulin curves have been calculated on the basis of hours Observation time is given in weeks

treatment of CHD but there has been little work on the effect of clofibrate on carbohydrate metabolism in CHD patients In the present study clofibrate treatment seems to improve the i v glucose tolerance and lower the insulin secretion after a glucose load However these patients all had a disturbance of glucose tolerance These effects and their relation to lipoprotein disturbances and their relevance to CHD patients with a normal GTT remain unclear The well known effects of clofibrate in lowering triglycerides (2 21) and fribrinogen (3) have been confirmed These effects do not correlate to the effect on glucose metabolism in the present study

Improved oral glucose tolerance has been demonstrated after clofibrate treatment by Ruzyllo et al (24) in CHD patients and clofibrate has also been shown to improve the glucose tolerance in juvenile (20) and postclimacteric (19) diabetes Ferrari et al (12) found improved glucose tolerance and reduced insulin secretion after 7 days of clofibrate treatment in patients with chemical diabetes and hypertriglyceridemia They suggested that the drug acts by removing some factor(s) other than triglyceride cholesterol or uric acid (possibly free fatty acids) predisposing to insulin resistance Ritland et al (23) reported lowered insulin in hyperlipidemic patients after glucose loading when treatment with the bile sequestering agent polidexide was combined with clofibrate Fendorson et al (11) also reported improved oral tolerance lowered insulin and reduced serum free fatty acid levels on clofibrate

The method used in our study for evaluating the glucose tolerance by calculating *k* values after i v glucose is well established (18) but shows rather large fluctuations in our patients as recently reported by other workers (14) This objection to the method has to some extent been overcome in this study by following the patients every fourth week for 24 weeks

Epstein (9) suggests that disturbed glucose tolerance elevated triglycerides and obesity may well form a metabolic syndrome in which a mechanism related to insulin plays a key role affecting atherosclerosis and CHD Our study confirms the beneficial effect of clofibrate on the abnormal glucose tolerance The improved glucose tolerance occurs in spite of a lowered insulin secretion after a glucose load which makes it probable that clofibrate acts in some way by reducing peripheral insulin resistance The importance of these effects of clofibrate in the treatment of CHD patients with impaired gluco[se] metabolism remains to be evaluated in prospecti[ve] studies

ACKNOWLEDGEMENTS

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A Family with Dominantly Inherited Mild Juvenile Diabetes

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ABSTRACT A family of 53 members is described in which mild diabetes in young, non obese subjects is transmitted through four generations from parents to children, the ratio diabetic/non diabetic offspring of diabetic parents being 3 2 and all affected individuals having a diabetic parent In this family, mild juvenile diabetes thus appears to be inherited as an autosomal Mendelian dominant

It has been known for centuries that diabetes mellitus is a familial disease Genetical investigations during the last 50 years have shown that genetic factors are of importance for the familial aggregation of the disease There is however still little agreement as to the mode of genetic transmission and different modes have been proposed for juvenile and maturity onset diabetes respectively

Mild diabetes in young people i e mild juvenile diabetes has been the subject of steadily growing interest although the disease was actually described as long ago as in 1798 by Rollo (4) Mild juvenile diabetes is characterized by no or only a few mild symptoms no ketonuria or insulin dependence In 1967 it was demonstrated that in patients with this type of diabetes as expected the blood insulin level could be increased after stimulation in contrast to classic juvenile diabetics (2) Since then several reports have appeared about mild juvenile diabetes mainly concerning the insulin response pattern The prevalence of the condition is completely unknown

In 1974 Tattersall (5) described three families in which mild juvenile diabetes appeared to be autosomal dominantly inherited and in 1975 Tattersall and Fajans (6) published an extensive study on the different inheritance of classic juvenile and mild juvenile diabetes in obese and non-obese subjects

It is the aim of this paper to describe a family in which obesity does not play a role for the accumulation of mild diabetes in young subjects but in which autosomal dominant inheritance is the most probable explanation for the familial aggregation of the disease

METHODS

All the family members answered a questionnaire If they claimed not to be diabetic their urine was tested with a Clinistix® (Ames Company) 2 hours after a meal Urine from spouses of diabetic persons was tested in the same way If this test was negative the subject was considered non-diabetic All the diabetics had been hospitalized and information about them was obtained from hospital records Patients with random blood sugar values below 200 mg/100 ml underwent an oral glucose tolerance test The result was defined as diabetic if the blood sugar was higher than 120 mg/100 ml 2 hours after the glucose challenge

RESULTS

Table I and Fig 1 show data on the diabetic family members Table II on the spouses of diabetic persons and on the non diabetic family members

No case of consanguinity was reported Seven of the 13 spouses of diabetics had their urine tested but none had glycosuria The family comprised 53 members of whom 22 (12 males 10 females) appeared to be diabetic (41%) Body weight of 18 of the 22 diabetics was known and only one was obese (Documenta Geigy Scientific tables 6th ed 196[illegible] >115% of average b wt) Fig 1 shows that dia[illegible]betes was diagnosed in all but one of the d[illegible] family members before the age of 20 From [illegible] I it appears that the symptoms were mild [illegible] and that the diagnosis in 15 cases (70 [illegible] accidentally by routine urine testin[illegible]

Table I *Clinical data*
+ = present − = absent

Family member	Year of birth	Duration of diabetes (y)	Presentation	Follow up: Average b wt (%)	Symptoms	Blood sugar (mg/100 ml)
1	1889	16	Thirst and sugarspots on the shoes	85	?	100–200
2	1910	44	Routine urine test	97	Thirst fatigue polyuria	200–300
3	1911	5	Visual disturbances hunger thirst genital pruritus		Diabetic coma	360
4	1912	23	Routine urine test		?	150–170 (fasting)
5	1914	18	Routine urine test	96	Tiredness headache thirst polyuria *furunculosis*	70–200
6	1915	32	Routine urine test	100	Thirst polyuria loss of weight fatigue genital pruritus	80–260
8	1920	34	Routine urine test	97	Fatigue thirst polyuria	175–225
12	1930	27	Routine urine test	105	Polyuria hunger	110–250
14	1933	3	Routine urine test	100	Thirst	80–225
15	1935	25	Thirst tiredness genital pruritus	110	None	90–140[a]
16	1943	4	Thirst loss of weight tiredness	90	Thirst hunger polyuria	90–160[a]
17	1946	3	Routine urine test	123	None	75–160[a]
19	1936	10	Polyuria tiredness furunculosis	107	None	90–170[a]
20	1951	4	Routine urine test	98	Malaise fatigue	100–200
21	1958	4	Routine urine test		None	100–200
26	1950	2	Fatigue	85	Fatigue	180–280
27	1957	1	?	92	Fatigue	200–270
30	1959	0	Routine urine test	105	None	120–230
31	1952	4	Routine urine test	109	None	135–220
36	1958	10	Routine urine test		Fatigue thirst	100–175
37	1964	2	Routine urine test		None	( )
43	1956	5	Routine urine test	114	Thirst polyuria	190–350

[a] Abnormal oral glucose tolerance test

were confirmed during follow up admissions Seven of the 22 patients did not have definitely elevated diurnal blood sugar concentrations but in every case the glucose tolerance test was clearly diabetic Glycosuria was present in all patients but only 6 (27%) had had ketonuria Seven of the patients had never required insulin treatment and the highest insulin dose given was 60 U/day Only six of the 15 insulin treated patients had received insulin immediately from the time of diagnosis The mean interval from diagnosis to start of insulin treatment was 7 years (range 1–19) Despite a duration of diabetes of 2–44 years (mean 13) very few signs of long term diabetic complications were found

Fig 1 shows that diabetes was directly transmitted from parents to children through four gen

Glycosuria (g/24 h)	Ketonuria	Max insulin requirement	Insulin started (y after diagn)	Other treatment	Complications
40	–	NPH 16	9 (28)	CH fixed diet	Died from coronary thrombosis at age 56
10–150	(+) Hunger	NPH 60	3	CH-fixed diet	1–2 retinal microaneurysms
Heavy	+	NPH 28	4	CH-fixed diet	Died in diabetic coma
100–180	+	None		Free diet minus sugar	?
0–90	–	NPH 16	18	CH fixed diet	?
18–1.4%	–	NPH 24	2		Peripheral arteriosclerosis
0–30	–	NPH 40	19	CH fixed diet	4–5 retinal microaneurysms neuropathy renal glycosuria
0–50	–	NPH 36	0	Clofibrate	Hyperlipoproteinaemia type IIb
5	+	NPH 40	1	CH fixed diet	?
10	–	NPH 36	0	CH fixed diet	None
0–25	–	None	0	CH fixed diet	None
0	–	None		CH fixed diet periodically chlorpropamide 250 mg	
1%	–	NPH 24	7	CH fixed diet	Foetus mortus anencephalicus (1959)
10–30	–	None		CH fixed diet	None
10–12	–	None		CH fixed diet	None
30–80	+	NPH 32	0	CH fixed diet	Hypercholesterolaemia
30–60	–	NPH 12	1	CH fixed diet	None
0	–	NPH 20	0	CH fixed diet	Hyperlipaemia type IV
10–40	–	NPH 20	(for 3 mo)	Tolbutamide 500 mg×3 1968–	None
10–70	–	None		Glibenclamide 5 mg×3	None
+					Renal glycosuria
	–	None		Free diet minus sugar	? renal glycosuria
30–90	+	NPH 28 mane Reg 12 NPH 12 vesp	0		None

erations and that the ratio diabetic/non-diabetic family members was 1 1.4 (22 31) among offspring of *affected* parents it was 3 2 (21 15)

DISCUSSION

The type of diabetes in this family differs from the usual pattern in young subjects. Classic juvenile diabetes is characterized by acute onset severe symptoms ketonuria and insulin dependence whereas in this family the typical picture is mild with few or no symptoms no ketonuria and no insulin dependence (1)

Fifteen of the 22 diabetics had no symptoms at all the hyperglycaemia was mild to moderate and only 27% had ketonuria. The maximal insulin dose

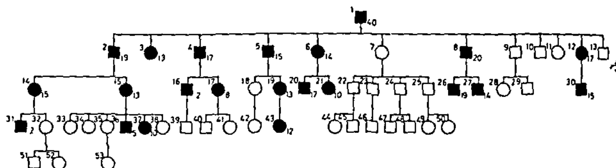


Fig 1 Pedigree of the family Non-diabetic=□ (male) ○ (female) diabetic=■ (male) ● (female) Figures to the left of the symbols=family member number to the right =age at diagnosis of diabetes

was 60 U/day and many of the insulin treated diabetics could probably have insulin substituted with oral antidiabetic drugs without altering the metabolic state

Every affected person had an affected parent and the ratio diabetic/non diabetic subjects was very close to the theoretically expected 1 1 in dominant inheritance

Thirteen of the diabetic family members were married It was however possible to get into contact with and to test the urine for glucose in only 7 of the spouses None of them had glycosuria

Obesity is often aggregated in families and is often accompanied by diabetes or glucose intolerance which disappears after weight loss (3) Mild diabetes in obese young subjects may therefore give a false impression of dominant inheritance due to familial occurrence of obesity

From the tables in the paper by Tattersall and Fajans (6) on the inheritance of mild juvenile diabetes it can be calculated that 23% of the diabetics and 24% of their siblings were obese In the present family information on body weight was available in 18 of 22 diabetics and only one was obese Therefore in this family obesity does not seem to play a role for the accumulation of diabetes

Further studies are in progress in our laboratory in order to elucidate whether mild diabetes in non obese young subjects is invariably genetically homogeneous Preliminary studies seem to indicate that this is not the case

Table II *Data on spouses of diabetic family members and non diabetic family members*

	Spouse			
Family member	Year of birth	Urine test for glucose	Family member	Year of birth
Diabetics			*Non diabetics*	
1	1889	–	7	1917
2	1909	–	9	1923
3	Unmarried	–	10	1927
4	–	–	11	1928
5	1901	–	13	–
6	1921	Neg	18	–
8	1921	Neg	22	1938
12	1920	Neg	23	1944
14	–	–	24	1945
15	1930	Neg	25	1940
16	1944	Neg	28	1954
17	1940	Neg	29	1959
19	–	–	32	1954
20	Unmarried	–	33	1952
21	Unmarried	–	34	1954
26	Unmarried		35	1956
27	Unmarried	–	38	1971
30	Unmarried	–	39	1967
31	1957	Neg	40	1971
36	Unmarried		41	1974
37	Unmarried	–	42	–
43	Unmarried	–	44	1961
			45	1963
			46	1962
			47	1968
			48	1973

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Prevalence of Angina Pectoris and Myocardial Infarction in a General Population Sample of Swedish Men

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ABSTRACT A primary preventive trial against cardiovascular diseases has been in progress since 1970 in Goteborg, Sweden The study population comprises all men in the city born in 1915–22 and 1924–25, a total of about 30000 men One third of this population constitutes the intervention group The prevalence of angina pectoris and myocardial infarction among men aged 47–53 years has been studied in this group The cases were identified by means of a postal questionnaire, direct interview at screening examination and by clinical examination A validation of the postal questionnaire technique gave a sensitivity of 74% and a specificity of 94% The minimum prevalence of angina pectoris amounted to 4 3% and the prevalence of myocardial infarction to 1 6% In the angina series, 22% had suffered a myocardial infarction that preceded angina pectoris in 9%

In Sweden as in most industrialized countries the disease pattern is dominated by cardiovascular diseases Among Swedish men aged 50–54 years ischemic heart diseases (IHD) account for 40% of deaths (17)

A primary preventive trial against cardiovascular diseases in men aged 47–55 years was initiated in 1970 in Goteborg This made it possible to study the occurrence of IHD in that age group The purpose was to study the prevalence of definite and suspected clinically defined angina pectoris as well as the prevalence of myocardial infarction in the above mentioned population In follow up studies risk factors and prognosis concerning angina pectoris will also be presented

STUDY POPULATION AND METHODS

Goteborg is a typical industrial and maritime city with 446000 inhabitants The study population comprises men residing in the city and born in 1915–22 and 1924–25 in all about 30000 individuals (20 21) Men born in 1923 were excluded because of participation in another study The 30000 men were divided randomly into three groups each comprising approximately 10000 men (Table I) One group (A) as well as a subsample of group B were sent an *illustrated postal questionnaire concerning heredity for* cardiovascular diseases smoking habits physical activity during work and leisure time stress known myocardial infarction known hypertension chest pain related to effort pain in the calves shortness of breath and production of phlegm Subjects belonging to group C were not contacted at all Among those who answered the postal questionnaire all subjects in group A—the intervention group—were invited to a screening examination in the afternoon

Height weight BP ECG and serum cholesterol were registered at screening The ECG registration was performed with standard leads and evaluated according to the Minnesota Code (1) In addition the participants were interviewed in more detail according to a questionnaire concerning previous diseases and present symptoms

The prevalence study of angina pectoris commenced with men born in 1917 and was then completed for all the subsequent age groups Identical examination of men born in 1915 and 1916 had made the physician confident of the method

The postal questionnaire concerning chest pain is a somewhat shortened version of Rose s questionnaire (13) for the diagnosis of ischemic heart pain in field surveys (Table II) The criteria for positive postal questionnaire angina were that the chest pain was brought on by physical exertion and also that it was relieved when the activity *was interrupted The men who fulfilled the criteria* were interviewed at the screening examination by a physician according to a more detailed questionnaire i e Rose s questionnaire with some additional questions (Table III) Physicians trained in this type of examination took part in the screening

Men who were judged to have definite or suspected angina pectoris were invited to another examination by a single physician (MH) This examination which was considered to be the final evaluation concerning the occurrence of angina pectoris comprised an interview according to the questionnaire heart auscultation determination of BP and ECG (11 lead)

For evaluation of the reliability of the postal question

Table I *Design of preventive study in Göteborg*

The study group comprises all men born in 1915–22 and 1924–25 (*N*=30 000) Group A=intervention group groups B and C=control groups (*N*≈10 000 each)

	Group A	Group B
Investigation	Postal questionnaire Heredity Heart symptoms Physical activity Occupation Leisure time Smoking Stress	Postal questionnaire Same as in group A
	Examination Height weight BP cholesterol ECG *Special questionnaires* and tests in subsamples	Examination *Same as in group A in a subsample*
Treatment	Risk factors treated High BP High cholesterol Smoking (Physical inactivity)	
Follow-up after 4 years	Postal questionnaire Check of results on risk factors	Postal questionnaire *Same as in group A in a subsample*
End-points	Deaths all causes Deaths cause specific Non fatal myocardial infarction Non fatal stroke	In groups A B and C

naire a random sample of the participants in group A (all whose date of birth ended with 1) were interviewed at the screening concerning the occurrence of chest pain whether or not they had mentioned such symptoms on the postal questionnaire

The screening started in Jan 1971 with men born in 1917 and was completed in March 1973 with men born in 1925 The clinical examination started in March 1971 and was completed in Oct 1973 except for occasional subjects who came to clinical examination as late as in Feb 1974

Registration of all subjects who have suffered a myocardial infarction has been going on in Göteborg since 1968 (4) Follow-up of these patients is taking place at a special postmyocardial infarction clinic—the Post MI Clinic (3 5 18) The subjects who were patients at the Post MI Clinic when entering the present study were only sent the postal questionnaire but not invited to screening Further information was collected from the records of the Post MI Clinic where the subjects had been assessed according to corresponding criteria and by the same team of physicians as in the primary preventive trial

In order to ascertain the prevalence of myocardial infarction in the series we also started out from the questions in the postal questionnaire concerning myocardial infarction (Table II) At the screening examination the same questions were presented at an interview The information was then checked against hospital records The same criteria as in the infarction register were required for the diagnosis

RESULTS

Total series

The number of subjects entering the present study was 8 092 (Table IV) The postal questionnaires were answered by 82 3% and 75% participated in the screening The ages of the participants ranged from 47 to 53 years (mean 50 3)

The criteria for postal questionnaire angina were fulfilled by 701 subjects of whom 647 agreed to participate in the screening On that occasion extra cardial factors were judged to account for the chest pain in almost half of the cases The average prevalence of definite and suspected angina pectoris was 5 7% (345 subjects) Of these 329 agreed to take part in the final clinical examination The evaluation of their symptoms is shown in Fig 1 At the final clinical examination the mean prevalence of definite angina pectoris was 2 9% and of suspected angina pectoris 1 4%

Table II *Postal questionnaire concerning chest pain*

Do you suffer pain or discomfort in the chest when you walk up slopes or stairs or when walking rapidly on the level?

Do you suffer pain or discomfort in the chest when walking at a normal pace on the level?

Questions in this section need to be answered by only those who answered one of the above questions affirmatively

If you suffer pain or discomfort in the chest in connection with effort do you usually
- Stop?
- Slow down?
- Continue at the same pace?

If you stop or slown down does the pain then disappear?

If it disappears how rapidly?
- After less than 10 min
- After more than 10 min

Where do you get this pain or discomfort?
(The participants are asked to mark with a cross in an illustration of the chest)

Have you ever suffered severe chest pain during half an hour or more?

Have you ever been hospitalized due to myocardial infarction?
- Which year?
- Which hospital?

Table III. *Rose's questionnaire with additional questions*

1 Have you ever had pain, pressure or tightness in your chest?
Yes 1 No=2 (If No stop here, if Yes answer the questions below.)

2 Have you got the impression that the pain is connected with effort?
Yes 1 No 2 (If No pass to question 12.)

3 Do you get it when walking uphill or climbing stairs or when you hurry on the level?
Yes 1 No 2 Never walk uphill or hurry 3

4 Do you get it when you walk at an ordinary pace on the level?
Yes 1 No 2

5 Do you get it when working hard with your arms?
Yes 1 No 2 Never work with my arms 3

6 Do you get it during exertion in cold or windy weather?
Yes 1 No 2 Never exert myself in cold or windy weather 3

7 What do you do if you get it?
Stop 1 Slow down 2 Carry on 3

8 If you stop, what happens?
Relieved 1 Not relieved 2

9 How soon?
Within 10 min or less 1 Within more than 10 min 2

10 Will you show me where you feel the pain?
Upper sternum 1 Mid sternum 2 Lower sternum 3 Left anterior chest 4

11 Does the pain radiate?
No radiation 1 Left arm 2 Right arm 3 Both arms 4 Neck 5 Arms and neck 6 Back 7 Abdomen 8

12 Have you had chest pain at rest?
No 1 When getting excited or angry 2 During worry and anxiety, stress 3 After meals 4 For no reason 5 At night 6 Combinations 7

13 How often do you get pain during the winter (Nov.–March)?
Never 1 Very occasionally 2 Several times a month 3 Several times a week 4 Daily 5

14 How often do you get pain during the summer (June–Sept.)?
Never=1 Very occasionally 2 Several times a month 3 Several times a week 4 Daily 5

15 For how long have you had this discomfort?
<2 years 1 2–5 years 2 >5 years 3

16 Chest pain—doctor's decision
No pain 1 Typical angina pectoris 2 Suspected angina pectoris 3 Suspected infarction 4 Other chest pain 5

17 *Myocardial infarction*
Have you ever had a severe pain across the front of your chest lasting for half an hour or more?
Yes 1 No 2

18 *Do you take tablets for*
High BP? Yes 1 No 2
The heart? Digitalis Yes 1 No 2
Nitroglycerin Yes 1 No 2
Other tablets Yes=1 No 2
High serum lipids? Yes 1 No 2

Do you take
Tranquilizers or sedatives? Yes 1 No=2
Other medicines? Yes 1 No 2

Evaluation of reliability of the postal questionnaire

In the subsample of group A used for evaluating the reliability of the postal questionnaire and comprising 966 subjects, the postal questionnaire was answered by 801 (83%) and 735 (76%) participated in the screening. The mean age was the same as in the total series, 50.3 years. At the screening also subjects who had denied chest pain in the postal questionnaire were interviewed concerning such symptoms according to the more detailed questionnaire described above (Table III). The prevalence of definite and suspected angina pectoris was then 9.1%. At the final clinical examination this figure was reduced to 5.9%, of which 2.6% were definite and 3.3% suspected angina pectoris.

Sensitivity and specificity

According to the postal questionnaire 65 subjects in the subsample fulfilled the criteria for angina pectoris and 59 of them attended the final clinical examination. Fourteen subjects were judged to have definite and 11 suspected angina pectoris. Adding these groups, 25 subjects were positive to the postal questionnaire as well as clinically. This means that the postal questionnaire gave 34 false positive cases (Table V).

Among the 676 men with no angina pectoris according to the postal questionnaire 5 had clinical angina pectoris and were consequently false negatives. There were also 13 cases of suspected angina pectoris among these 676 men. If they are added to the 5 cases of definite angina pectoris, the number of false negative cases becomes 18.

The sensitivity, by which is meant the percentage of clinically positive angina pectoris identified as positive by the postal questionnaire, amounts to 74% if the cases with suspected angina pectoris are judged as negative and 58% if they are judged as positive.

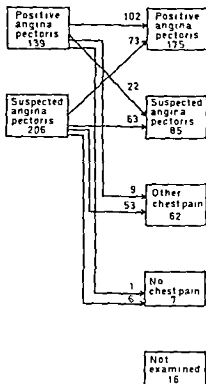


Fig 1 Subjects with positive and suspected angina pectoris at the screening examination and at clinical evaluation

The specificity i e the percentage of clinically judged negatives identified as negative by the postal questionnaire is 94% and 95% respectively

The prevalence of myocardial infarction Angina pectoris associated with myocardial infarction

In the postal questionnaire 155 men reported that they had suffered a myocardial infarction Of these men 150 attended the screening examination and at interview it was possible to rule out myocardial in farction in 17 cases Checking the patient records however revealed that another 36 subjects had not sustained a myocardial infarction but had been treated in hospital because of other diagnoses Summing up it was not possible to verify sustained myocardial infarction in 53 out of the 150 cases

Ninety seven subjects (1 6%) had suffered a myocardial infarction 3 months–18 years before the screening examination

Pain typical of angina pectoris during more than one month had been experienced before the myo cardial infarction by 46 (47%) of the 97 cases Among these 46 subjects 12 (26%) became free from angina pectoris after the myocardial infarc tion In 24 cases angina pectoris made its first appearance after the myocardial infarction and in 27 cases (28%) the patients with myocardial infarc tion were quite free from a history of angina pectoris Table VI summarizes the different sub-groups of men with angina pectoris and myocardial infarction

DISCUSSION

Patients with angina pectoris and myocardial infarc tion have an increased risk of early death and some studies indicate that the prognosis in men with angina pectoris is almost the same as following myocardial infarction (8 11, 24) It is important to establish by means of population studies the pre-valence and prognosis of angina pectoris within age groups in which an improvement of the prognosis with respect to death rate and invalidism may be expected with medical or surgical treatment

A main problem in all population studies is the achievement of a sufficiently high participation rate This is likely to depend on the one hand on previous contacts between the individual and the health and medical service of the community and on the other on the extent to which information concerning the investigation can be brought to the attention of the population

The non participant group in the present study has been analyzed It was found that both the mortality rate and the total morbidity were higher and social and alcoholic problems were more com mon among non participants than participants (19)

Table IV *Number and percentage of subjects with angina pectoris according to postal question naire screening and clinical examination*

	N	%	
Postal questionnaire sent	8 092	100	
Attended screening examination	6 070	75	
Postal questionnaire angina in the screening population	647	10 7	
Definite angina pectoris at screening examination	139	2.3	5 7
Suspected angina pectoris at screening examination	206	3 4	
Definite angina pectoris at clinical examination	175	2 9	4 3
Suspected angina pectoris at clinical examination	85	1 4	

Table V *Distribution of cases by clinical evaluation and postal questionnaire response*

Figures within parentheses denote cases with suspected angina pectoris summed with positive cases

Postal questionnaire response	Clinical evaluation of angina pectoris		
	Positive	Negative	Total
Positive	14 (25)	45 (34)	59
Negative	5 (18)	671 (658)	676
Total	19 (43)	716 (692)	735

Whether or not angina pectoris was more frequent in the non participant group is not known but a check of hospital records did not point in that direction

Rose (13) found that questionnaire technique gave a relatively high sensitivity (about 83%) and high specificity compared with diagnosis on the basis of clinical examination In the Evans County study (7) Rose s questionnaire gave a sensitivity of 81% and a specificity of 97% The questionnaire was then used for direct interview by trained personnel and individual variation between different interviewers was assumed to be negligible In the present study however postal questionnaire technique and a shortened version of the questionnaire had to be used which caused a lower sensitivity 74% (58%) The specificity however was high 94% (95%) Obviously occasional individuals with angina pectoris did not account for their symptoms or they reported chest pain but did not fulfill the criteria for a positive diagnosis e g by denying the correlation to effort

The prevalence of angina pectoris in the population has previously been reported from the USA (2) Denmark (6) and Finland (12) The population samples in these studies were smaller than in the present series and only in the Finnish study was population sampling done from the official census Some age groups and occupational categories were overrepresented The series differed considerably with respect to the account of various manifestations of IHD In the Framingham study (2) the concept of atherosclerotic heart disease was used as a composite term for myocardial infarction angina pectoris acute cardiac death obviously caused by coronary heart disease and ECG manifestations of sustained myocardial infarction and myocardial fibrosis The prevalence of atherosclerotic heart disease was 4 6% in ages 45–62 and 3 7% in ages 45–54 The prevalence of definite angina pectoris was 1 9% (18 of 941 men) In the Evans County Study (7) the prevalence of angina pectoris among 768 white men aged 22–78 was 4 1%

Tibblin (15) reported a prevalence of angina pectoris of 2 1% among 50-year old men in Göteborg and of 5 5% among men aged 54 The Copenhagen study (6) comprising more than 5 000 male employees aged 40–59 showed a prevalence of angina pectoris of 3 1% In Finland where the morbidity rate from IHD is considerably higher than in Sweden the corresponding figure among men aged 55–59 was 8 2% (12) The prevalence of angina pectoris in the present study (4 3%) might be regarded as a minimum At direct interview the control group presented a prevalence of 5 9% but it is notable that the group which increased at interview was that of suspected angina pectoris The prevalence of definite angina pectoris was about the same in the two series

Owing to the variability in the symptomatology of angina pectoris it is important not to base the diagnosis on data from a single examination The chance of identifying the angina pectoris patient by means of a postal questionnaire or by a single interview obviously increases if the symptomatology is typical and the attack rate high Rose has confirmed that the point prevalence underestimates the true prevalence to a degree that is proportional to the variability of the characteristics of symptoms (14) For certain evaluation of the prevalence several investigations within a relative short period are required as the risk of new cases appearing increases with the length of the observation time Individuals who have previously suffered discomfort but have improved have a tendency to neglect their symptoms Similar findings were reported in the Israeli

Table VI *Prevalence of angina pectoris (AP) and myocardial infarction (MI)*

	n	% of all 6 070 subjects	% of subjects with AP
AP without MI	202	3.3	78
AP before and after MI	34	0 6	13
AP after MI	24	0 4	9
AP before but not after MI	12	0 2	
MI without AP before or after MI	27	0 4	

study (10) showing that at re-examination in 1968 almost 20% of individuals with angina pectoris in 1963 denied that they had ever had chest pain In the present study also some men who had first reported chest pain later denied this symptom

The prevalence of myocardial infarction was 1 6% in the present study and 1 4% at age 40–59 in the Copenhagen study (6) while in Finland it was 6 7% at age 55–59 (12) The Framingham study reported a prevalence of 1 6% at age 45–62 (2) It is not possible to draw any definite conclusions from these figures because of selection of the series and divergences in age groups Another well controlled study (4) has shown a true difference in the prevalence of myocardial infarction between Helsinki and Goteborg

It is notable that 1/3 of men who reported that they had suffered a myocardial infarction had in reality been treated for another disease (angina pectoris pericarditis valvular heart disease arrhythmias virus infections gallbladder disease and deforming spondylitis) Obviously information concerning the cause of the symptoms had been faulty In many cases the patient had become obsessed with the fact that he was admitted for observation for myocardial infarction It is important to try to decrease the iatrogenic injuries that this may bring about

Angina pectoris developed after the infarction in 25% of those in the present study who had had a myocardial infarction compared with 31% among the men in the Framingham study (8) Twelve (26%) of 46 became free from their angina pectoris after the infarction compared with 4 (15%) of 29 men in the Framingham Study The former figure approaches what is assumed to be a spontaneous regression of angina pectoris without intervening infarction viz about 30% It should be observed that the results from Framingham extend over an age range of 30–75 years

In the present study angina pectoris developed after myocardial infarction in 9% of the total angina series This may be compared with 47% among men and 15% among women in the Framingham study Thus in this respect the figures for angina pectoris among Swedish men seem to correspond to those among American women and interestingly enough this is also applicable to the incidence figures for myocardial infarction

The prevalence figures for myocardial infarction and angina pectoris among men in the Framingham study of 1949–52 are of about the same magnitu as those in the Study of Men Born in 1913 Göteborg of 1963 It is unreasonable to comp the results of the present study directly with t results from Framingham in so far as the Frami ham figures go 20 years back in the past Av able mortality statistics reveal a considera higher infarction rate in the USA and Finland th in Sweden This is also reflected in a higher mort ity rate for IHD Thus in 1971 the mortality in t group of disorders among men aged 55–64 ye was about twice as high in Finland as in Swe and the mortality figures for the USA are alm comparable with the Finnish ones (22 23) The di ferences cannot be completely explained by dif ences in actual figures for risk factors between different countries (9) However the prevalence smokers is lower in Sweden than in both Finl and the USA It is interesting that in the 1970s USA and Finland had a cigarette consumption capita and year that was not reached in Swe until 1971 (16)

ACKNOWLEDGEMENTS

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Atenolol in the Treatment of Angina Pectoris

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STRACT Nineteen men aged 41–64 years with ble angina pectoris have completed a random double blind study of atenolol 50 mg b i d atenolol 100 b i d and placebo Fifteen patients had subjec improvement on atenolol two were unchanged two felt worse (because of asthenia/leg fatigue) significant placebo effect was found On both nolol dosages there were highly significant reduc ns in heart rate at rest and during exercise and BP Only the maximal heart rate decreased signifi ntly more on 100 mg atenolol than on 50 mg ($p <$ 01) Fourteen patients had the same or a better ysical performance on the 50 mg b i d regimen an on the 100 mg b i d regimen although this dif rence was not significant Sixteen patients had high bicycle exercise performance on atenolol than on acebo Disregarding the three non responders a ean increase of 44% in bicycle performance was und No serious side effects were seen Most indi iduals reported an increased feeling of well being on tenolol

n recent years β adrenergic blocking agents (β lockers) have been used extensively in the treat ent of angina pectoris (? 7) Atenolol (Tenormin) as been used for some time as an anti hypertensive gent but to judge from the literature experience ith this drug in the treatment of ischaemic heart isease is almost negligible

In an earlier paper (3) we have presented evi ence suggesting a probable beneficial effect of tenolol in the treatment of angina pectoris using dosages far below those recommended hitherto The present study was started in order to assess the effects of atenolol on the subjective and objective effort tolerance and quality of life in individuals with stable angina pectoris

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MATERIAL AND METHODS

Twenty patients with a mean age of 54.3 years (range 41 64 S D 6 3) were selected for this double-blind randomly assigned study They were selected provided they fulfilled the following criteria Presence of typical angina pectoris for at least 1 year being stable for at least 3 months prior to the trial During the baseline examination angina and/ or a positive exercise ECG had to be elicited during a bicycle exercise test to permit assessment and quantification of any change in effort tolerance during the exercise test to come

Patients with cardiac failure necessitating digitalis and/ or diuretics were excluded as were those treated with antarrhythmics those with 2nd or 3rd degree AV block bundle branch block diabetes mellitus treated with insulin or severe peripheral artery insufficiency

Obstructive airway disease (in two cases) resting pulse rate below 50 (one) previous myocardial infarction (four) and cardiac enlargement (one) were not considered absolute contraindications but particular care was taken in such cases

The subjects were outpatients treated by J D and K O for at least one year in all instances in most cases far longer They were known to be reliable in taking prescribed medicines and they were all thought to be mentally stable They were fully informed about the procedures except that they were told they would receive the drug in 4 different doses each dose given for 3 weeks During the first 3 weeks all patients received a placebo The trial proper consisted of three 3 week periods during which each patient received each of the following treatments according to a random Latin square design placebo atenolol 50 mg b i d atenolol 100 mg b i d Identical looking tablets of placebo and atenolol were supplied by ICI Pharmaceuticals Division Macclesfield England who also performed the randomization

Initially and at the end of each 3 week period the following examination was made clinical examination with recording of symptoms resting systolic blood pressure (SPB) resting pulse rate and possible signs of heart failure In addition patients were asked about possible side effects and their opinion on the effects of the drug The results were graded crudely as worse unchanged 0 definite improvement + considerable improvement/free from angina ++ All had free access to medical supervision and control during the trial

Peak expiratory flow was measured with a Wright s

Table I *Subjective vs objective improvement during the various trial periods (i e subjective drug preference vs highest performance on bicycle)*

Objective preference	Subjective preference: Placebo	Indifferent (=no change)	Atenolol 50 mg×2	Atenolol 100 mg×2	Total
Placebo	1			2	3
Atenolol					
50 mg×2	1		7*	1	9
100 mg×2		2	1	4*	7
Total	2	2	8	7	19

* Patients with concordance between subjective and objective effect

peak flow meter An exercise test was performed on an electrically braked Elema bicycle starting with a load of 300 kpm (≈50 W) and with a stepwise increase by 300 kpm (≈50 W) every 4 min Details about leads used methods of recording during and after exercise and general supervision are presented elsewhere (3) The exercise was continued until terminated by angina fatigue or dyspnoea or the occurrence of an ST depression of 3 mm The reason(s) for discontinuing the test was recorded individually and common safeguards were used during the test In theory the trial was double blind but of course at examination any β-blocking effect was easily disclosed

The individuals were instructed to take the drug at exactly the same time in the morning and particular care was taken to ensure that the exercise tests were performed at exactly the same time of the day Using the baseline test time as the reference all other tests in all individuals were performed within ± 10 min of that test time

All clinical examinations and judgement of the subjective effect were assessed independently of the exercise test and in all instances the clinical assessment preceded the exercise test Patients were not allowed to eat or smoke during the last 2 hours before the test which took place within 3–6 hours of the ingestion of the drug in all instances

RESULTS

One patient was excluded during the run in period because he developed atrial fibrillation All the others fulfilled the complete trial without complications One man had rib fracture during his placebo period II and could therefore not perform the exercise test at the end of this period

Table I shows the subjective vs objective preference of the various drug regimens Two patients complained of deterioration while on atenolol and two noted no difference between the four periods A complete subjective/objective concordance was found in 12 cases i e 7 showed more or less discordance between subjective effects and objective measurements as assessed by the bicycle exercise test

Fig 1 shows that in 8 of the 19 patients the 50 mg×2 dosage gave the highest exercise performance In 6 patients the two atenolol dosages were equal in this respect and only 5 (of whom 2 weighed approximately 100 kg) had a higher performance on 100 mg×2 Thus 50 mg atenolol was equal to or better than 100 mg in 14 of the 19 cases

Table II indicates the number of patients who had or did not have angina severe leg fatigue and/or a positive exercise ECG during and/or after exercise Almost identical results were found during the baseline examination and the two placebo examinations During both atenolol periods, significantly fewer had angina during the tests Simultaneously however significantly more patients discontinued the exercise tests due to leg fatigue as the main reason The number of ECGs with ST depressions remained roughly unchanged during all periods

Table III shows the exercise parameters obtained during the five exercise tests As mentioned above one of the patients had a rib fracture during placebo period II If we extrapolate from baseline and pre

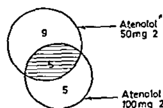


Fig 1 Venn diagram presenting the relation between highest exercise performance and dosage of atenolol * = highest performance on the dosages indicated

Table II *Presence (+)/absence (−) of angina pec‑ toris (A), leg fatigue (F) and significant ST depres‑ sion (ST) during the various periods of treatment*

	A+*	A−	F+**	F−	ST+***	ST−
Baseline						
examination	16	3	1	18	10	9
Placebo						
Period I	16	3	2	17	11	8
Period II*	17	1	2	16	10	8
Atenolol						
50 mg×2	11	8	9	10	10	9
100 mg×2	8	11	11	8	8	11

One individual had a rib fracture during placebo period II and could not perform the exercise test
Difference in occurrence rate during placebo vs atenolol *p=0 0014 ** p≤0 001 ***p≥0 10

vious placebo average data for this individual approximately two beats in maximal pulse rate during exercise and approximately 300 kpm should be added to the mean value for cumulative work during placebo period II (i e the placebo period during the trial proper) Provided that this extrapolation is valid there are no significant differences in any of the exercise parameters during the three non-drug tests There are highly significant reductions in resting pulse rate maximal heart rate and maximal BP during the two atenolol periods as compared with placebo and also a significant increase in cumulative work performed There is a significantly lower maximal pulse rate on atenolol 100 mg×2 vs atenolol 50 mg×2 while the differences concerning resting pulse rate maximal BP and cumulative work are not significant

The grading of the subjective effects on angina symptoms is indicated in Table IV Two patients deteriorated subjectively on atenolol Fifteen reported no effect of placebo even though they all thought they were taking an active drug during all trial periods

Peak expiratory flow averaged 605 l/min on placebo and 596 l/min during atenolol therapy The difference is not significant

Fig 2 shows the exercise performance in the individual patient in the two atenolol periods vs the mean baseline/placebo performance It is seen that 8 of the 10 individuals with the highest performance did less well on the higher dose of atenolol In the 9 individuals with the lowest performance 6 had a similar or better performance on the higher than on the lower dose

BP showed a consistent fall during atenolol therapy both in normotensives and in hypertensives A) Resting SBP was almost identical at the three non atenolol examinations mean 141 6 mmHg (S D 19 7) B) On atenolol 50 mg×2 SBP was 128 9 mmHg (S D 20 4) C) On atenolol 100 mg×2 SBP was 124 2 mmHg (S D 17 0) Differences (paired *t* test) A–B $p<0.01$ A–C $p<0.001$ B–C $p>0.10$

A few patients complained spontaneously of leg fatigue particularly on the higher dose of atenolol and some had a feeling of malaise when initiating β blocker therapy No case of asthma or cardiac failure occurred Most individuals reported cold hands on inquiry No nightmares were recorded Three patients reported colicky abdominal pain while on atenolol 100 mg b i d but not on 50 mg b i d The general trend however was that most individuals reported an increasing feeling of well being while on atenolol In particular no rebound effect was seen i e none reported a sudden and

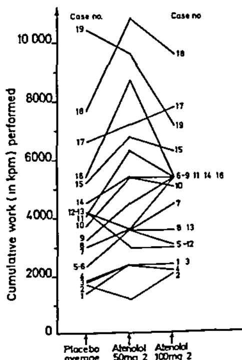


Fig 2 Individual variation in physical performance (cumulative work during bicycle exercise test) on placebo and two atenolol dosages in 19 men with angina pectoris

Table III *Data from bicycle exercise test during five tests in 19 men with angina pectoris*

x=mean value of each parameter S D =standard deviation of x=probability of significant differences in mean a: groups

	Resting pulse rate (beats/min)				Maximal heart rate during exercise (beats/min)				Maximal blood pressu during exercise (mmH			
	x	S D	p		x	S D	p		x	S D	p	
Baseline data	73 9	10 2			138 1	20 5			202	36		
Placebo												
Period I	74 3	9 5	NS	<0 001	138 4	18 3	NS	<0 001	200	31	NS	<
Period II	71 2	9 2			132 4	16 8			193	32		
Atenolol												
50 mg×2	56 2	8 3	NS		108 1	13 2	<0 01		171	30	NS	
100 mg×2	53 7	7 2			100 8	13 2			168	29		

NS=not significant (p>0 10)

severe deterioration in angina symptoms on switching from atenolol to placebo

DISCUSSION

Most β blockers have an effect on both β_1 and β_2 receptors Practolol has been shown to be a selective β_1 blocker for practical purposes (5) but because of its adverse effects another β blocker with the same degree of selectivity has been sought Atenolol has been shown to possess a high degree of β_1 receptor selectivity and is devoid of intrinsic sympaticomimetic activity and membrane stabilizing effect (1) Theoretically these properties should make atenolol a suitable β blocker in the treatment of angina pectoris as indicated in an earlier report on hypertensive patients (3) However we have found only one paper on the effect of atenolol in long term treatment of angina (8) In this study atenolol caused a highly significant drop in resting heart rate exercise heart rate exercise BP and hence pulse rate/BP product in all patients tested This was reflected in a better exercise performance in 16 of the 19 individuals of the present study during β blockade In 14 the performance on the 50 mg dosage was equal to or better than that on the 100 mg dosage This indicates that in this population 50 mg should be the standard dose and that only a relatively small proportion of similar angina patients might expect a better effect on a higher dosage Whether a still lower dose migh be effective remains to be seen However it is noteworthy that we have found previously that approximately 1/3 of the individuals in an unselected group of hypertensives had an excellent BP lowering effec 25 mg atenolol b i d (3)

Little has been reported in the literature on possible deterioration of physical performanc higher dosages of β blockers such as indicate this study However we have had a similar ex ence with another commercially available drug dolol) in the same patients as in the present s (4), and a similar effect is also suggested in a re trial of alprenolol (6) Concerning atenolol the effective dose may be as low as 50 mg b i d as one of the 10 subjects with the highest physical formance had an improved effort tolerance on mg b i d In these individuals leg fatigue was m the limiting factor However in the individuals the lowest physical performance angina was limiting factor and it is conceivable that higher ages of β blockers may have a still better effe such individuals

Table IV *Clinical grading of effects of plac atenolol 50 mg×2 and atenolol 100 mg×2 on gina symptoms*

–=deterioration during therapy 0=no effect of ther +=definite improvement during therapy ++=consi able improvement during therapy and/or complete moval of angina symptoms

	–	0	+	++
Placebo	5	10	3	1
Atenolol 50 mg×2	1	5	8	5
Atenolol 100 mg×2	2	4	6	7

Cumulative work performed during exercise (kpm)			
	S D	p	
079	2 571		
126	2 328	NS	=0 002
667	2 057		
000	2 596	NS	
684	2 134		

Åstrom and Vallin (12) found effects similar to ours in their study of i v administered atenolol while Roy et al (8) found only a negligible improvement of experimental exercise tolerance in 11 patients with severe angina They were however patients at the extreme end of the angina spectrum and the experience from that study must be judged with caution The discordance between the results of Roy et al and ours may be explained partly by a different exercise protocol partly by a different dosage schedule

Estimating the anti anginal effect of a β blocker by means of nitroglycerin consumption may give unreliable results (9) An improvement in exercise tolerance may for instance cause the individual to perform more work and thus the frequency of angina attacks may be unchanged despite a real improvement The subjective feeling of improvement/deterioration is probably a better indicator of the effect Subjective improvement was reported in the present series by 15 individuals on atenolol objective by 16 However in 7 of the 19 individuals the subjective and objective effects were more or less discordant

There are several obvious reasons for such a discordance in a trial like ours 1) β blocker related leg fatigue (which is particularly likely to occur during the highly artificial strain on the bicycle) 2) marginal changes only noticed during the exercise tests 3) steep increase in load during the exercise test (which may hide clinically important improvement) 4) changing physical activity 5) changing weather conditions 6) training effect and 7) change in cardiac pathology Such confounding variables explain why we probably never will achieve complete subjective/objective agreement in any anti angina trial with β blockers

A few points need emphasis 1) The lack of placebo effect in our study is probably explained by the fact that the individuals were in a stable doctor–patient relationship 2) A training effect is for practical purposes excluded as analysis of variance showed no time trend in physical performance 3) We carefully ensured that the drug/exercise test time relationship was constant As all tests were performed within 3–6 hours after the ingestion of the drug an optimal serum concentration ought to have been present in most individuals during the test These facts suggest that the reported findings are real

The average improvement in bicycle performance in this study was 25% on the 50 mg×2 regimen Disregarding the 3 individuals who showed deterioration on both atenolol dosages the 16 responders had a mean *optimal* improvement of 44% an improvement which is statistically highly significant

As suggested by Tremblay et al (9) any drug trial should include scrutiny of the data to see if two or more populations exist showing qualitatively different reactions to the drug among a group of patients thought to be homogeneous In a trial like this we may divide the total group into responders and non responders and obtain a truer picture of mean response in those reacting favourably to the drug Why some of our individuals respond favourably and others do not remains speculative in the absence of haemodynamic data However β blockers are known to influence several of the factors which determine the myocardial oxygen consumption some favourably and some unfavourably and of course the clinical result depends on the sum of all these effects (2)

We conclude that atenolol is a safe and effective β blocker in the treatment of angina pectoris The negligible effect on peak expiratory flow in our patients is in good agreement with previous experience of the drug in individuals with obstructive airways disease (11 10)

In most instances there has been a subjective/objective concordance concerning the effect of therapy and in most instances the individuals also have an increased feeling of well being during atenolol therapy Few side effects were seen in keeping

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Swallowing Syncope

A Case Report

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ABSTRACT A previously healthy man complained of syncopes and convulsions during meals Despite thorough gastroenterologic, neurologic and cardiologic examination the condition was misinterpreted as a psychiatric disorder for 13 years Then ambulatory ECG, using a casette recorder, revealed periods of 2nd degree AV block during swallowing Pacemaker implantation prevented the attacks

In recent years interest has grown in ambulatory ECG for diagnosis and treatment of cardiac arrhythmias (3) This has attracted increasing attention to and knowledge of cardial causes of cerebral symptoms (2) Abdon and Malmcrona (1) used the term cardiogenic neurology for these conditions and noted a high rate of pacemaker implantation following systematized active diagnosis by means of long term ECG screening

The equipment for ambulatory ECG recording used in the present case is a casette tape recorder (A/S *Vingmed Recard) attached to precordial* leads The recorder may be switched on manually by the patient at onset of symptoms The tape recording after transcription is used for manual ECG reading

CASE REPORT

Man born in April 1915 A skiing accident at the age of 22 involved commotio cerebri without sequelae Otherwise he had no history of any remarkable illness especially no rheumatic fever or diphtheria

In 1963 (at the age of 48) he experienced syncopes of short duration while having his ordinary meals At that time he had no convulsions but prior to fainting he felt a tense boring pain in his back between the scapulae Symptom free periods lasted for days or weeks The symptoms were totally absent when he was not eating or swallowing

In 1967 after having consulted a general practitioner he was admitted to a specialist in neurology Examination including EEG did not reveal any explanation of the symptoms A psychiatric condition was suggested and the *patient was treated with ataraxics*

His symptoms persisted and he was hospitalized in 1970 A thorough examination was performed including X ray of the esophagus and upper gastrointestinal tract EEG and ECG ECG was registered under standard conditions at work load and while eating No pathology was detected Once more he was given ataraxics without any benefit In 1972 the same procedure was repeated now including full otolaryngologic status and X ray of the skull No further progress was made

Late in 1975 13 years after the first attack his symptoms became more frequent and serious He often fell to the floor became pale sometimes cyanotic and had generalized convulsions Still the attacks occurred only while eating Often his wife had to hold a hand behind his neck during meals to prevent him from falling A new hospital stay and thorough cardiologic and neurologic examination were again unsuccessful The cardiologic examination this time also included continuous ECG monitoring in the Coronary Care Unit for 48 hours

The patient was then given the tape recorder After two weeks at home he was able to present a tape which he had recorded while having a light attack Part of the recording is shown in Fig 1 It reveals an AV block of 2nd degree Despite previous attempts as mentioned above this was the first dysrhythmia to be recorded Occurring coincident with the attack of syncope a causal connection was most likely

The diagnosis of swallowing or deglutition syncope seemed to be a clear indication for pacemaker implantation He was given an on-demand pacemaker (Cordis Omni Stanicor) first programmed for a frequency of 80 later 60 impulses/min The benefit of the pacemaker is illustrated in Fig 2 This ECG recorded while the patient is swallowing shows a short period of AV block with pacemaker escape rhythm The swallowing is sometimes still painful but there have not been any further syncopes

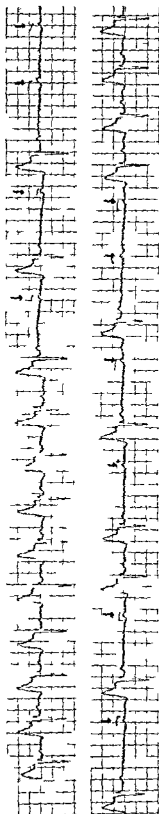


Fig 1

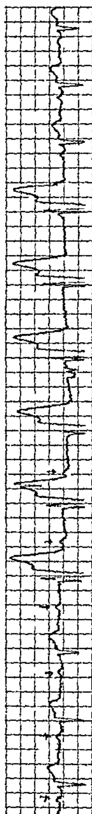


Fig 2

COMMENTS

The cause of the AV block in this patient probably has to do with vagal overload on a hypersensit or in some way damaged conducting system The etiology of the dysfunction of the conducting system is not clear but ischemic disease seems most reasonable although the patient had no other signs of ischemic heart disease or generalized arteriosclerosis No local changes in the esophagus and upper gastrointestinal tract were demonstrated which could account for the vagal "overactivity" This agrees with the experience of others (4) The patient had no sinus bradycardia orthostatic hypotension or other signs of vagotonic hyperactivity

The benefit of pacemaker implantation in this certainly life threatening condition is obvious

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Fig 1 Continuous recordings of the attack during swallowing which led to the diagnosis Arrows at non conducted P waves

Fig 2 Short period of pacemaker rhythm registered while swallowing Arrows at conducted and non-conducted P waves

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Primary Purulent Meningococcal Pericarditis with Tamponade

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ABSTRACT A case of primary purulent meningococcal pericarditis presenting with hemodynamic derangement due to tamponade, is reported Treatment was successfully fulfilled with antibiotics and surgical drainage using continuous percutaneous subxiphoidal catheter drainage with the Seldinger technique Less than ten cases of this potentially lethal entity have been reported in the literature

Pericarditis is an uncommon but well documented complication of meningococcemia and meningococcal meningitis Purulent meningococcal pericarditis in the absence of clinically evident meningitis is very much less common with only eight cases previously recorded (2–7 9 11) The present paper reports another patient in whom continuous percutaneous drainage of the pericardium was successfully used as a most valuable adjunct in the management

CASE REPORT

A 16-year-old female was admitted on May 22 1976 with diffuse abdominal pain of two days duration The pain was colicy and accompanied by yellow green fouly smelling diarrhoea She had no nausea and did not vomit Pulse 120 temperature 37 5 C BP 120/70 mmHg Hb 13 7 g/100 ml WBC 12 100/mm³ ESR 30 mm/h Clinically and radiologically peritonitis was diagnosed and laparotomy decided upon A pint of bloody fluid was found in the peritoneum and a ruptured follicle cyst in the left ovarium was resected

Postoperatively the patient was much more ill than one would expect after an operation of this magnitude The second postoperative day she became critically ill with frequent and shallow respiration clammy cyanotic skin tachycardia and hypotension Friction rub was heard over the sternum X ray of thorax disclosed pleural fluid bilaterally most on the left side Pulmonary infiltrations were present as well as enlargement of the heart ECG showed changes compatible with myocarditis Blood gas values revealed a severe hypoxemia and the patient was put on respirator with positive end expiratory pressure Blood was drawn for culture pleural drainage was established bilaterally and pericardiocentesis via the subxiphoidal approach yielded 450 ml purulent yellow fluid 400 ml of similar fluid was drained from the pleura The central venous pressure fell thereafter from 20–25 cm of water to 8–9 cm of water and the BP rose Microscopic examination of a stained specimen disclosed gram negative diplococci which by culture were found to be Neisseria meningitidis The patient was given Cedilanid Lasix and large doses of penicillin and Garamycin

On the day after the pericardiocentesis the heart was radiologically still enlarged and a 7 F polyethylene catether with end and side holes was placed with the Seldinger technique in the pericardium via the subxiphoidal route It was left in situ for three days and a total of 370 ml fluid was drained At first it was purulent later serous

The patient recovered slowly and after 10 days the respirator treatment could be terminated The patient was discharged after 4 weeks in hospital and at the last follow up on Sept 3 1976 she was well without any complaints The temperature ESR and ECG were normal as was X ray of the thorax

DISCUSSION

Early in the course of meningococcemia hematogenous seeding of the pericardium may occur resulting in bacterial invasion and purulent pericarditis which accounts for 4% of all cases of purulent pericarditis Pericarditis may also occur late in the course of meningococcal infection in patients treated with antibiotics and otherwise recovering In these patients hypersensitivity mechanisms producing sterile inflammation appear to i

pericarditis and they respond to antiinflammatory agents rather than to antibiotics

In the present case, fever and chest pain were rapidly followed by cardiac tamponade and hemodynamic derangement The patient had not received antibiotics and Neisseria meningitidis was cultured from the blood as well as from pleural and pericardial fluid In patients receiving antibiotics cultures may be negative The etiologic diagnosis may then be established with counter current immunoelectrophoresis (9) Identification of the responsible pathogen is of paramount importance so that proper antibiotics may be given

Our patient exhibited ECG evidence of myocarditis, another well recognized complication of severe meningococcal infection However throughout the illness she was free of cutaneous stigmata of meningococcemia and had no laboratory evidence of disseminated intravascular coagulation nor did she exhibit symptoms or signs of meningeal irritation A lumbar puncture was not performed because in the presence of purulent meningococcal pericarditis the findings of abnormalities in the cerebrospinal fluid would not have altered her therapy

The clinical diagnosis of purulent pericarditis is difficult and a delayed diagnosis is still one of the most important reasons for the high mortality (8) Plain X ray of thorax angiocardiography technetium scanning and echocardiography may be important supplementary diagnostic aids However in the critically ill patient a subdiaphragmatic pericardiocentesis must be performed without delay to establish an etiologic diagnosis and to institute the mandatory surgical drainage

The treatment of these patients must be an aggressive approach by a team of specialists Vigorous appropriate antimicrobial therapy should be given by parenteral routes Intrapericardial ins tion of antibiotics is unnecessary, as the antim bial agents show excellent penetration into per dial fluid (10) The surgical drainage must be quate Open drainage however is seldom essary A percutaneous subxiphoidal contin drainage with the Seldinger technique as ir present case, will obviate this This simple and technique has also been successfully used in ur hemopericardium (1) This procedure will se adequate drainage If the aspiration of fluid is ficult because of clotting the position of the ca ter in the pericardium may easily be change means of a curved guide wire Furthermore p sure readings as well as lavage and contrast in tion are easily undertaken to test the efficacy o *treatment*

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A Case of Severe Compression of the Coeliac Artery

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ACT A man, aged 42 years presented as an gency with a history of stabbing upper abdomi nausea and diarrhoea of two weeks' dura Apart from abnormal transaminase and al e phosphatase values the routine clinical and atory examinations did not reveal any abnor y As the abdominal pain increased in severity ularly in association with eating, and projectile ing supervened aortography was carried out howed severe stenosis of the coeliac axis, involv bout 1 cm of the artery At operation a thick us band, which originated from the median ite diaphragm, was seen to constrict the point of of the artery and to compress the vessel against iorta The band was divided whereupon the ic artery immediately showed strong pulsations idequate filling Microscopic examination of the us band revealed, among other structures, those icteristic of a ganglion The symptoms disap d after operation

ie great majority of cases in the West dis nce of blood flow in abdominal or other ies are secondary to arteriosclerotic vascular se or to cardiac disease associated with atrial ation and arterial embolism (5) Even in pa with other symptoms than those typical of emia the possibility of constriction of an ab nal artery should be considered In exception il complete cure can be achieved The case ted below is an illustrative example

CASE REPORT

atient a male chief mate aged 42 years presented emergency with a history of upper abdominal pain of ks duration The pain radiated to the back on both and had gradually increased in severity His father rom a cerebral vascular lesion otherwise there was own family history of vascular disease At the age of 33 years the patient had a gastrointestinal infection in Egypt About 10 years ago he had had catarrhal cystitis

About 2 weeks before the present admission the patient became ill with pain in the upper abdomen nausea and diarrhoea but did not vomit After 5 days rest and treat ment with antacids his condition improved One week before admission he suddenly perspired felt ill and fainted in the street He had no abdominal pain did not void urine and did not pass a stool During the two weeks before admission he noticed that his stools were offensive slightly looser than normal and discoloured being some times black and sometimes white His appetite had been good and bowels normal until 2 weeks before admission He drank alcohol only occasionally There was no history of venereal disease In view of the fact that the stabbing upper abdominal pain had become increasingly severe particularly in association with eating he attended the Casualty Department of this hospital and was admitted for observation

On admission he was found to be in good condition At palpation of the heart the impulse over the left ventricle was neither broadly increased nor heaved The heart sounds were essentially normal There was no bellows sound The carotid artery the radial artery the posterior tibial and dorsal arteries of the foot were equally distinctly palpable on both sides BP was 120 mmHg systolic and 80 diastolic in the right arm and 115 mmHg systolic and 70 diastolic in the left The abdomen was soft but tender over the epigastrium down to the umbilicus No pathological swellings were palpated The liver was palpated 2 cm breadth below the right costal margin Rectal palpation did not disclose any abnormality Digital measurement of the BI demonstrated evidence of generalized arteriosclerotic changes ranging from slight to moderate in the right leg

ESR was 11 mm/h ASAT 1 15 ALAT 2 57 and alkaline phosphatase 9 2 μkat/l (normal value 5 3) After one week in hospital the transaminase and alkaline phospha tase values reverted to normal Diastase in the urine and the duodenal secretion were normal Routine examination of the blood and the test for xylose yielded normal values Serum electrolytes and creatinine levels were normal The bromsulphalein test showed that the patient had retained 7% of the amount injected after 45 min Possible presence of a tropical disease was investigated in Roslagstull Hospi tal Stockholm with special reference to worm eggs and cysts but the results were negative Serology with



Fig 1 Aortography showing a coeliac artery with a proximal stenosis of about 1 cm (arrow) behind the superior mesenteric artery The lumen of the coeliac artery is only 1 mm in its narrowest segment

reference to leishmaniasis amoebiasis and trichinosis did not reveal any abnormality Sternal puncture disclosed a normal bone marrow and liver biopsy a normal parenchyma Cholecystography demonstrated normal filling and emptying No gallstones were visible There was no calcification in the region of the pancreas Scintigram of the liver showed uniform distribution of the isotope in the liver and spleen which were both of normal size X ray of the digestive tract did not disclose any pathological changes in the stomach duodenal bulb or colon Opaque medium was seen to pass at a normal rate through the latter Radiographic examination showed a normal heart size and no evidence of changes was found in the lungs Urography revealed the kidneys to be normal in size and there was no evidence of either delayed excretion or calcification Treatment with antacids resulted in an improvement in the patient s condition and he was discharged essentially symptom free

After about two months he experienced pain of a cramp-like nature in the upper abdomen which lasted for 1–3 hours The pain became increasingly related to taking food It commenced after eating and its severity was to some extent related to the size of the meal The patient had thrombophlebitis on three occasions thought to be due to previous parenteral feeding He was readmitted He developed transient eosinophilia Microscopy sho[w] veins to be rather dilated and their walls to be thi[c] the appearance suggesting the results of thromboph When projectile vomiting supervened aortograp[hy] carried out (Fig 1) and showed stenosis of the axis involving about 1 cm of its lumen the na[rrowing] being so severe that only about 1 mm of the lum[en] left Neither the hepatic nor the splenic arteries [showed] any changes They filled via anastomoses betw[een the] latter two arteries and the superior mesenteric arte[ry]

Summarizing the findings there was severe ste[nosis of] the coeliac artery and collateral filling of the h[epatic] splenic and superior mesenteric arteries On acc[ount of] these findings the patient was referred to the Depa[rtment] of Surgery for reconstructive surgery Expl[orative] laparotomy was carried out The pancreas st[omach] spleen liver and gallbladder appeared normal Con[centrat]ing at the lesser omentum above the stomach the [dissec]tion was performed in such a way that the upper [part of] the aorta was partly exposed However the coeliac [artery] was not accessible by this route and it was neces[sary to] dissect between the colon and stomach instead Th[e] ligament was divided the pancreas and stomac[h] lifted up and in this way it was possible to exp[ose the] superior mesenteric artery The latter was rema[rkably] thickened and showed weak pulsations A thrill was [felt in] the hepatic artery It was unduly wide and thi[s was] thought to be due to stenosis The coeliac arte[ry was] dissected free as far as the aorta and here a thick [fibrous] band was seen to constrict the point of origin of the [artery] and to compress the vessel against the aorta Th[e band] originated from the median arcuate ligam nt of [the dia]phragm It was divided whereupon the coeliac art[ery im]mediately showed strong pulsations and adequate [...] The thrill in the hepatic artery vanished after com[pletion] of the dissection

During the first postoperative weeks the patien[t occa]sionally experienced pain which radiated to t[he] shoulder Otherwise the postoperative course w[as un]eventful At the follow up examination 6 week[s post]operatively he was completely symptom free a[nd had] returned to his previous activities

Histological examination of a liver biopsy take[n at op]eration showed a fairly large amount of connective [tissue] in some portal areas and a slightly increased num[ber of] lymphocytes The fibrous band that had constric[ted the] vessel contained connective tissue and numerous [...] cells with the microscopic appearance of a ganglio[n]

DISCUSSION

In the case presented a median arcuate ligam[ent of] the diaphragm was found to constrict the po[int of] origin of the coeliac artery

Dunbar et al (2) were the first to describe [...] of localized constriction of the coeliac arter[y by the] ligament of the diaphragm by fibrosis of the c[oeliac] axis or by an arcuate ligament transformed [...] ganglion Thereafter about 120 similar cases

…orted (4) Harjola and Lahtiharju (3) de… …8 cases of localized constriction of the …rtery The average age of their patients was … They and other investigators have stres… …localized constriction of the coeliac artery …d with diffuse symptoms

…esenting symptoms in the patient reported … diffuse upper abdominal pain nausea and …a These symptoms were initially thought due to *acute gastritis* and treatment with … was in fact followed by an improvement in …ent s condition However the symptoms …ently increased in severity and as there was …ease in serum transaminase and alkaline …tase *cholecystitis* was suspected How… …olecystography did not reveal any abnor… In view of the fact that the patient had been … in tropical countries and that the …phalein test suggested liver damage it was …ed important to rule out *parasitic dis…* …uch as trichinosis amoebiasis and leish… … Eosinophilia swollen and tender veins …ed the possibility of *periarteritis nodosa* … other symptoms of this condition were … As projectile vomiting developed the pos… … of *pancreatitis or a neoplasm of the* …s was considered but could not be con… For the same reason an *obstruction high up* *gastrointestinal tract* was considered but …f the digestive tract showed that the opaque … passed normally through the colon As pain … increasingly related to meals constriction of an artery in the upper part of the abdomen was considered Aortography was therefore carried out and disclosed a fibrous band which was subsequently divided surgically

The constricting structure may be a ganglion or a fibrous band the so-called *coeliac axis syndrome* Owing to the development of an extensive collateral circulation the syndrome may not cause any symptoms until arteriosclerosis develops which may e… sue at an early stage Other less common aetiological factors responsible for arterial insufficiency in upper abdominal organs are localized artherio sclerotic dissection of the aorta luetic aortitis arteriovenous fistulae neoplastic infiltration of arteries and Takayasus disease (1)

The aim of reporting this case was to draw attention to the fact that this condition which is amenable to treatment may be the cause of upper abdominal pain of uncertain origin

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ANNOUNCEMENTS

XIV International Congress on Therapeutics will be held in Montpellier France Sept 8–10 1977

Administrative secretariat Midi contact 8 rue d Alger B P 1041 F 34006 Montpellier Cedex France

International Symposium on Somatostatin a satellite symposium to the 13th Annual Meeting of the European Association for the Study of Diabetes (EASD) will be organized in Freiburg/Br W Germany immediately preceding the EASD congress Sept 25–27 1977 Sponsored by the Serono Research Foundation Europe Inc Congress language is English

Information S Raptis M D Department of Internal Medicine Endocrinology and Metabolism University of Ulm Steinhoevelstrasse 9 D 7900 Ulm Germany or J E Gerich M D Metabolic Unit 1143 HSW University of California Medical Center San Francisco Calif 94143 USA

International Conference on Atherosclerosis sponsored by the European Group for the Study of Atherosclerosis and the Italian Society for Atherosclerosis Research organized by the Lorenzini Foundation of Milan will take place in Milan Nov 9–11 1977

The congress will be divided into the following sessions Atherosclerosis and Heart Atherosclerosis and Brain Atherosclerosis and Peripheral Circulation

Chairmen of the meeting L Carlson Stockholm R Paoletti Milan G Weber Siena

Information Fondazione G Lorenzini Via Monte Napoleone 23 Milan Italy

Neuer Preis gestiftet Die Boehringer Ingel uka GmbH hat den Eugen Werle Preis ge DM 10 000 – dotiert ist Ziel des Preises rung der Diagnostik auf dem Gebiet der Kl mie und der Klinischen Mikrobiologie Um sich jeder Wissenschaftler mit einer deuts noch nicht veroffentlichten methodischen gen Die Arbeit muß zu einer wesentlich diagnostischen Aussage fuhren Der Preis Jahre anlaßlich der Medica durch den Pr Kongresses erstmals 1977 verliehen Der halt neben der Dotierung eine Urkunde

Fur die Verleihung 1977 soll die Arbeit Ausfertigung bis zum 31 07 77 an den Stift Herrn Dr med R Berensmann Genera Deutschen Gesellschaft zur Förderung der Diagnostik Jahnstraße 32 Arztehaus D-7 70 eingesandt werden Das Manuskript selbs Namen und keinen Hinweis auf den Autoren Arbeit ist mit einem Kennwort zu versehen satzlichen verschlossenen Umschlag der da der Arbeit tragen muß sind anzugeben V name Beruf Stellung und Tatigkeit Ad Kennwort der Arbeit

Mit Einreichen der Arbeit erklart sich d der Veroffentlichung einer Kurzfassung in d Diagnostik einverstanden Das Preisric besteht aus namhaften unabhangigen Wiss Bei der Preisvergabe ist der Rechtsweg au